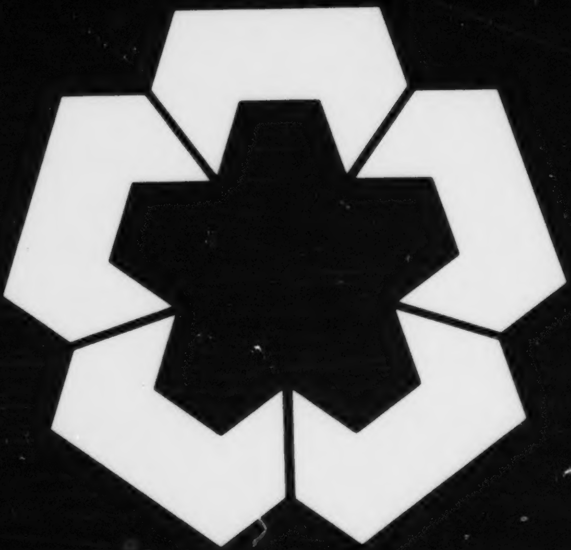


Potential Health Problems Caused by Disposal to Land of Sewage Sludge Containing Pathogens

RMD Report 83 21

Alberta

The logo represents the interdisciplinary and holistic nature of the Division's environmental research programs. Each of the five sections of the symbol represent comprehensive programs on air, water, land, humans, and their constant interaction. The sections converge to form a cohesive chain, characteristic of an effective research organization. This cohesive bonding makes the sections blossom into the environment in the form of a flower — an Albertan wild rose.

POTENTIAL HEALTH PROBLEMS CAUSED BY DISPOSAL TO LAND OF SEWAGE SLUDGE CONTAINING PATHOGENS

by

DONALD L. LEHMANN
PETER M. WALLIS
ARLENE MacMILLAN
JUDITH M. BUCHANAN-MAPPIN

Kananaskis Centre for Environmental Research
The University of Calgary

for

RESEARCH MANAGEMENT DIVISION
Alberta Environment

FINAL REPORT
RMD 83/21

June 1983

For information on reports published by the Research Management Division, contact:

Research Management Division
14th Floor, Standard Life Centre
10405 - Jasper Avenue
Edmonton, Alberta
T5J 3N4

(403) 427-3946

This report may be cited as:

Lehmann, D.L., P.M. Wallis, A. MacMillan, and J.M. Buchanan-Mappin. 1983. Potential health problems caused by disposal to land of sewage sludge containing pathogens. Prep. for the Research Management Division by Kananaskis Centre for Environmental Research, Universit of Calgary. RMD Report 83/21. 172 pp.

POTENTIAL HEALTH PROBLEMS CAUSED BY DISPOSAL TO LAND OF SEWAGE SLUDGE CONTAINING PATHOGENS

RESEARCH MANAGEMENT DIVISION
Alberta Environment

This report is made available as a public service. The Alberta Department of Environment neither approves nor disagrees with the conclusions expressed herein, which are the responsibility of the authors.

TABLE OF CONTENTS

Page

LIST OF TABLES ix

LIST OF FIGURES xi

EXECUTIVE SUMMARY xii

ACKNOWLEDGEMENTS xix

1. INTRODUCTION 1

2. LITERATURE SEARCH: PARASITOLOGY 4
2.1 Influence of Sewage Treatment on Parasites 5
2.1.1 Primary Treatment 5
2.1.2 Sludge Systems 6
2.1.3 Effect of Physico-chemical Treatment on Parasites 8
2.1.4 Lagoons 8
2.2 Survival of Parasite Stages on Food Plants and Fodder 9
2.3 Sources and Presence of Parasites on Sludged Fields 11
2.4 Distribution of Parasite Eggs in Soil 11
2.5 Parasite Stages on Land 12
2.5.1 Infectivity of Parasites 14

3. LITERATURE SEARCH: THE BACTERIA 18
3.1 Bacteria from Sewage and Its By-Products: An Overview 18
3.2 Survival of Pathogenic Bacteria on Soil and Sludged Soil 27
3.2.1 Survival of Indicator Organisms on Soil 27
3.2.2 Survival of _Salmonella_ spp. on Soil 28
3.2.3 Survival of Other Pathogens in Soil or Sludge . 30
3.2.4 Growth on Soil and Mechanisms of Destruction .. 30
3.2.5 Adsorption of Bacteria by Soil 33
3.2.6 The Secondary Pathogens 34
3.2.7 Numbers of Other Pathogens in Sludge 39
3.2.7.1 _Mycobacterium_ 39
3.2.7.2 _Staphylococcus_ 39
3.2.7.3 _Pseudomonas_ 39
3.2.8 Numbers of Bacteria Required to Infect 40

4. METHODS 41
4.1 Sampling Locations 41
4.2 Methods of Sampling 42
4.3 Methods: Parasites 42
4.3.1 Parasite Analysis 42
4.3.1.1 Raw Sewage and Effluent 43

TABLE OF CONTENTS(CONTINUED)

Page

4.3.1.2 Raw and Anaerobically Digested Sludge 43
4.3.1.3 Lagoon Sludge 44
4.3.1.4 Soil-Sludge Mixtures 44
4.3.1.5 Analysis for Infective Larvae of Parasitic Nematodes 46
4.4 Methods: Bacteriology 48
4.4.1 Indicator Organisms and Enteric Pathogens 48
4.4.1.1 Total Coliforms, Most Probable Number (MPN) Method 48
4.4.1.2 Faecal Coliforms (MPN) 48
4.4.1.3 Faecal Streptococci (MPN) 49
4.4.1.4 Faecal Streptococci (Pour Plate Method) 49
4.4.1.5 *Clostridium perfringens* 49
4.4.1.6 Enteric Pathogens 49
4.4.2 Primary and Secondary Bacterial Pathogen Analyses 50
4.4.2.1 Sampling 50
4.4.2.2 Total Plate Count 50
4.4.2.3 MacConkey Plate Count 50
4.4.2.4 Identification of the Enterobacteriaceae 52
4.4.2.5 *Pseudomonas* Analysis (MPN) 53
4.4.2.6 *Staphylococcus* Analysis 54
4.4.2.7 *Mycobacterium* Analysis 54
4.4.2.8 Faecal Coliforms 54

5. RESULTS AND DISCUSSION: PARASITES 55
5.1 Crossfield 55
5.1.1 The Crossfield Lagoon System 55
5.1.2 Investigations at a Barnyard, Pasture, and a Spray Irrigated Field 59
5.2 Identification of Eggs From the Slaughterhouse and Sludged Fields 61
5.3 The Survival and Hatching of Parasitic Nematode Eggs in Various Media 64
5.4 The Influence of Drying of Worm Eggs 72
5.5 The Bonnybrook Facility: Raw Sewage and Digesters. 75
5.6 Worm Eggs Recovered from Fields Sludged in 1978, 1979, and 1980 76
5.6.1 Vertical Distribution of Worm Eggs: the 1980, 1979, and 1978 Sludged Fields 76
5.6.2 Spatial Distribution of Worm Eggs on the 1980 Sludged Field 78
5.7 Viability of Parasite Eggs on the 1978 to 1981 Sludged Fields 83
5.8 The 1981 Field Before and After Sludging 84
5.9 The 1982 Sludged Field 88
5.10 The Pasture 89
5.11 The Control Field 89
5.12 Raw, Anaerobic Digester, and Lagoon Sludge 90

TABLE OF CONTENTS (CONTINUED)

Page

5.13 Buried/Injected Sludge 90

6. RESULTS AND DISCUSSION: BACTERIA 92
6.1 Crossfield 92
6.1.1 The Crossfield Sewage Lagoons: Faecal Coliforms and *Salmonella* 92
6.1.2 The Barnyard and Pasture near Crossfield 96
6.2 The Sludged Fields, Control Field, and Pasture ... 96
6.2.1 The Faecal Coliforms 96
6.2.2 The Total Coliforms 100
6.2.3 The Secondary Pathogens 108
6.2.4 The Numbers of the Enterobacteriaceae: MacConkey Plate Counts 111
6.2.5 The Primary Pathogens 113
6.2.5.1 *Salmonella* 113
6.2.5.2 *Staphylococcus* 113
6.2.5.3 *Mycobacterium* 114
6.2.6 Fluorescent Pseudomonas 114
6.3 The 1982 Sludged Field 116
6.4 Bacterial Analyses of Raw, Digested, and Lagoon Sludge from the Bonnybrook and Fish Creek Plants . 120
6.4.1 Faecal Coliforms 120
6.4.2 Total Coliforms 120
6.4.3 The Secondary Pathogens 121
6.4.4 The Numbers of the Enterobacteriaceae: MacConkey Plate Counts 121
6.4.5 The Primary Pathogens 121
6.4.6 Fluorescent Pseudomonas 121
6.5 Buried/Injected Sludge 121
6.6 The Viruses 127

7. GENERAL DISCUSSION 131
7.1 The 1982 Field 131
7.2 The 1980 and 1981 Sludged Fields, the Control Field, and the Pasture............................ 131
7.2.1 The Parasites 132
7.2.2 Faecal Coliforms (MPN) 132
7.2.3 The Total (Indicator) Coliforms 132
7.2.4 Secondary Pathogens 133
7.2.5 Total Numbers of the Enterobacteriaceae: Allergens.................................... 134
7.2.6 The Primary Pathogens 134
7.2.7 Fluorescent Pseudomonas 135
7.2.8 The Viruses 135
7.2.9 Other Organisms 135
7.3 Raw, Mesophilic Digester, and Lagoon Sludge 136
7.4 Buried/Injected Sludge 138

8. CONCLUSIONS 141

TABLE OF CONTENTS(CONCLUDED)

Page

9. RECOMMENDATIONS AND SUGGESTIONS 145
9.1 Recommendations 145
9.2 Suggestions for Further Research 146

10. REFERENCES CITED 147

LIST OF TABLES

Page

1. Survival Times of Various Parasites on Plants and Soil 10

2. Survival Times of Various Bacteria on Soil, Plants, and in Water 21

3. Survival of Pathogenic Bacteria on Soil 29

4. Average Percentages of Enterobacteriaceae from Digested Sludge 37

5. Percentages of Pathogens Present in Effluent Spray Irrigation Aerosols 38

6. Crossfield Parasitology Examinations 56

7. Parasitological Results from the Barnyard and Pasture 60

8. Eggs from the Faeces of Slaughterhouse Animals ... 62

9. Infective Strongyle Larvae Hatched from Slaughterhouse Faeces 63

10. Infective Larvae Found from Sludged Fields Excluding Ascarids 65

11. Parasites Found Presumed Capable of Infecting Man (According to Literature Sources) 67

12. Effects of Drying on Worm Eggs 74

13. Vertical Distribution of Parasite Eggs on Disced Sludged Fields 79

14. Parasite Eggs Found in Raw, Digested, and Lagoon Sludge in April 1982 91

15. Crossfield Lagoon System: Faecal Coliform Analysis. 93

16. Results of the Bacteriological Analysis of Bottom Sludge Around the 12-Month Lagoon 94

17. Crossfield: Faecal Coliforms 97

18. Faecal Coliforms Isolated by MPN 98

19. Genera of Bacteria Isolated by Season from Sludged Fields, Control Field, and Pasture 103

LIST OF TABLES (CONCLUDED)

Page

20. Percentage of Coliforms of Total Isolates Made by Kott (1977) from Wastewaters 106

21. Numbers of Total Coliforms (ECE) 107

22. Comparison of ECE from Digesters, Digested Sludge, and Sludged Fields 109

23. The Sludged Fields: MacConkey Agar Counts and Percentages of Oxidase Negative Colonies 112

24. Isolation of Acid Fast Bacteria from the Fields .. 115

25. 1982 Surface Sludged Plot 117

26. Genera of Bacteria from Sludge 119

27. Microbiological Data from Bonnybrook and Fish Creek Sludge 122

28. Hand-Buried Sludge 124

29. Injected Sludge 126

LIST OF FIGURES

Page

1. Baerman Apparatus 47

2. Crossfield: Twelve-Month Lagoon Sampling Points and Numbers of Worm Eggs per kg Sludge 57

3. Crossfield Lagoons 58

4. Horse Parasite Stages in Manure 68

5. Horse Parasite Stages: Manure Mixed with Sludged Soil 69

6. Horse Parasite Stages: Manure Mixed with Lagoon Sludge 70

7. Average Number of Worm Eggs in Shepard Lagoon Sludge and Sludged Fields 77

8. Distribution of Parasite Eggs on the 1980 Sludged Field (Untransformed Data) 81

9. Distribution of Parasite Eggs on the 1980 Sludged Field (Transformed Data) 82

10. Survival of Helminth Eggs on Sludged Fields 85

11. Survival of Helminth Eggs on Sludged Fields (Data Transformed to Natural Logarithms) 86

12. Crossfield: Twelve-Month Lagoon Sampling Points and Faecal Coliform Numbers per 100 mL Sludge 95

13. ECE Percentages from the Sludged Fields and Pasture 101

EXECUTIVE SUMMARY

This document constitutes the third and final report of the project. Included are certain investigatory aspects from previous years because they are considered important unto themselves, or are essential to the understanding of the work accomplished during the final year. In addition to the experimental data from the final year of the project, other information is included, specifically:

1. meteorology , both bacteriological and parasitological;
2. bacterial and parasite literature reviews updated from those found in the First Annual Report (Lehmann et al. 1981); and
3. information pertaining specifically to the work done on sludged fields over the past three years.

The literature review and methodology dealing with viruses from sludge and sludged land remains in the Second Annual Report (Lehmann et al. 1982) and is not reproduced herein. The discussion is followed by a section of Conclusions, and the document is terminated with a series of recommendations with regard to the placing of sludge on agricultural fields and the use of such fields afterward.

The lagoon system at Crossfield is efficient in the removal of Salmonella and the reduction of faecal coliforms (MPN) to 20/100 mL in the 12-month lagoon. Parasite eggs (mainly strongyles) are concentrated in the bottom deposit of the 12-month lagoon and their placement is a function of water current and prevailing wind pattern. A field spray-irrigated with effluent from the 12-month pond showed neither parasite eggs nor faecal coliforms.

Domestic animals contribute substantially to the parasite (and presumably the bacterial) load of sludge. Fourteen species of parasitic worms were found from cattle, hogs, and horses at a Calgary slaughterhouse. In addition to the eggs of ascarids and Trichuris (which are readily identified), strongyles constituted the bulk of the species. Because of similarity in shape (and overlapping sizes) they must be hatched in order to identify the infective larvae. Some of the larvae, as well as the ascarids and

Trichuris, have been found from sludged fields. Eggs or larvae of 11 species capable of infecting man have been recovered from sludged fields.

The eggs of parasitic worms are adversely influenced by dessication while those of free living nematodes (which may resemble those of parasites and hatching to yield infective larvae often presents the only method of authentication) are more resistant. Strongyle eggs placed in lagoon sludge show a survival rate in excess of 90% over a 31 day period but remain static and begin to develop only when exposed to air. When mixed with soil, about 90% of strongyle eggs are killed over a 31 day period but the remainder hatch and develop into infective larvae.

When sludge containing parasite eggs is disced into the soil, 85% of the eggs are found in the top 2.5 cm after less than one year; 13% were noted in the mid 5 to 7.5 cm level, and less than 2% above the hard-pan at the 10 to 15 cm level. After 2 and 3 years the percentage of eggs at the top level is reduced and the total number of eggs also diminishes radically. It is presumed that fungi, soil invertebrates, possibly bacteria, and the nature of the soil are responsible for the reduction of numbers. The spatial (horizontal) distribution of eggs in the top 2.5 cm is not random. Instead, pockets or concentrations of eggs were noted, presumably the result of varying amounts of sludge on the surface as well as the tendancy of eggs to sediment in lagoon sludge prior to disposal causing uneven distributions. There is a rapid mortality of worm eggs after sludge deposition and discing, and low levels of eggs on fields planted shortly after sludging/discing are reached within 18 to 20 months.

Prior to the third year of the study, it became evident that proving an actual health hazard from sludged fields was a difficult if not impossible task. Instead of attempting to prove the existence of a health hazard, it was decided to look for conditions approaching those found on an unsludged, control field. In addition, investigations were conducted on a pasture to determine if it exceeded the potential for hazard as compared with both the sludged fields and the control field. The parameters selected for investigation were (1) the numbers of parasite stages; (2) the numbers and percentages of total coliforms; (3) the presence or absence of viruses; (4) the presence or absence of faecal coliforms; (5) the presence or absence of certain primary pathogens; (6) the presence of secondary pathogens (Enterobacteriaceae), the numbers and the percentage of each species; (7) the total numbers of the Enterobacteriaceae which could be equated with their potential to act as allergens; and (8) the presence or absence of fluorescent pseudomonads.

The 1980 and the 1981 sludged fields were selected for further study. The fields differed substantially from fields sludged in 1978 and 1979 in that the 1980 sludged field was disced and immediately planted and the 1981 field was sludged, disced, and allowed to lie fallow until 1982 when it was planted. The control field was planted with hay and had not been grazed for at least three years but the pasture was grazed by cattle during the study period.

Parasite stages disappeared from the 1981 field a few months after application of sludge. Small numbers of parasites were found from the control field, presumably from resident deer, and the numbers of eggs from the 1980 sludged field were similar to the control after 21 months. The numbers of eggs contributed by cattle and deer to the pasture were double those found on the control field .

Faecal coliforms appeared erratically, tending to disappear during winter months and reappear, at times, in warmer months. Fluorescent pseudomonads were also enigmatic and appeared only during the fall. Primary pathogens were not found.

The numbers and percentages of indicator (total) coliforms from the pasture were twice as great as from the sludged and control fields. *Escherichia coli* was found rarely from the control field, more frequently from the pasture, and not at all from the sludged fields. The total numbers of the Enterobacteriaceae were low during the winter, increased dramatically during the spring, and fell to levels somewhat above those found in the winter during the summer and fall. Less than an order of magnitude difference was noted, with the control field being lowest. The numbers of indicator bacteria (except for *E. coli*) rose proportionally during the spring, implying that the total coliforms are capable of reproduction on sludged land, pasture, and control fields. The presence of *E. coli* from the control field and the pasture is attributed to ongoing contamination by wild and domestic animals.

An analysis of the relative percentages of secondary pathogens (Enterobacteriaceae) revealed that almost 60% of the isolates were those which yield the smallest number of secondary infections (15%) according to the Center for Disease Control. E. coli and Proteus/Providencia cause two-thirds of secondary infections and E. coli was not found from sludged fields but it was isolated rarely from the control field and more frequently from the pasture. Proteus/Providencia was recovered only from the pasture. Viruses were not found from sludged fields, lagoon sludge, pasture, or the control field.

Sludge placed on grass (the 1982 field) shows a complete loss of parasite stages within 44 days. Shortly after sludging, dessication reduces the numbers of the Enterobacteriaceae; however, following summer rains, the numbers recover but to numbers appreciably less than those found in sludge. In the warmer months, the percentage of species is similar to those noted in lagoon sludge, except for Proteus/Providencia which disappear within 43 days. Colder weather brings about a reduction in numbers and a modification in species composition. Winter samples are similar to the control field.

Hand-buried sludge, undisturbed for 258 days, displayed a decrease in the number of parasite stages from 17 000 to 220 eggs/kg dry wt. After 258 days, the numbers of Enterobacteriaceae were small, 2000 x 10^4/g dry wt., of which about half were total coliforms. Proteus/Providencia were not isolated but E. coli were present in the amount of 267 x 10^4/g dry wt.

Injected sludge was examined one week after placement and in excess of 10 000 eggs/kg dry wt. and 260 000 x 10^4/g dry wt. Enterobacteriaceae were noted. Shortly thereafter, the field was sludged, disced, and allowed to lie fallow for the summer after which it was again disced and planted with winter rye. Since the injected sludge was fragmented and disbursed by discing, no attempts were made to search for the material; instead, surface samples, where sludge had been displaced, were examined. Fifty-five days after the first discing, no parasite eggs were found and none were found subsequently, even after the second discing.

Proteus/Providencia were never found from the surface. After the first discing, the numbers of Enterobacteriaceae and the total coliforms fell to sludged/control field level, though 5×10^4 *E. coli*/g dry wt. were recovered. The second discing recontaminated the surface with Enterobacteriaceae but at a level three to four orders of magnitude less than noted in the injected sludge. Sixty days later, the bacterial constituency resembled the sludged and control fields. *E. coli* were not isolated.

Raw, digester, and lagoon sludge were investigated. The numbers of parasite stages in digester sludge were more than quadrupled over the numbers in raw sludge. The numbers theoretically should have doubled and a possible explanation is that the eggs tended to sediment out and concentrate toward the point of sampling. The numbers of eggs from lagoon sludge were similar to digester sludge; on the basis of dry weights, the numbers from the lagoon should have about quadrupled. It is presumed that eggs are destroyed by lagooning over time, though those remaining are static and viable.

Anaerobic, mesophilic digestion reduced the numbers of Enterobacteriaceae in raw sludge by 89 to 99% but the relative proportions of genera remained similar with *E. coli* and *Proteus/Providencia* predominating. In lagoon sludge, the total numbers of Enterobacteriaceae increased so as to exceed the numbers from raw sludge and the proportions of genera remained the same. It appears, then, that the Enterobacteriaceae, including all of the total coliforms, are capable of growth in lagoon sludge. When sludge is deposited on land, conditions (biological and physical) are such that the secondary pathogens of greatest disease significance (*E. coli* and *Proteus/Providencia*) are removed while the remainder of the Enterobacteriaceae are capable of survival and growth when conditions become suitable. *Salmonella* were found from raw and digester sludge but not from lagoon sludge.

The conclusions are based upon the evidence uncovered during the investigation and reinforced, in certain instances, by published material cited in the literature reviews and in the body of the text. These items are numerous and should be consulted in their entirety.

The recommendations are derived from the material in the body of the text which is condensed in the section entitled Conclusions. The recommendations are:

1. Effluent from the 12-month lagoon at Crossfield may be used for spray irrigation of fodder crops:
    (A) one month of warm weather (July to mid-September) should elapse after spraying before animals are allowed to graze; and,
    (B) If hay is to be taken from the field, one month of warm weather (July to mid-September) should intervene between spraying and harvesting.
2. Surface sludged and disced land should be allowed to remain fallow throughout the following winter after which cereal or oil crops may be planted; animals may be allowed to graze on any plant residue following the harvest.
3. Winter cereal crops may be planted in the fall following surface sludging, discing, and fallowing. Animals may consume the plant residue the following year after harvesting.
4. If market gardening (truck farming) is contemplated (i.e., cultivation of leafy vegetables, root crops, cole vegetables, legumes etc.), the following regime should be followed. The field after sludging, discing, and fallowing over winter should be planted the next spring with cereal or oil crops. The following year (two years after sludging), consumable vegetables may be planted. If fall cereal crops are planted in the same year as sludging/discing/fallowing, the crops should be harvested and the field allowed to fallow through the second winter after which consumable crops may be planted.
5. For injected sludge, the same recommendations as noted in 2, 3, and 4 should be followed.
6. If sludge is applied to pasture, it should be done so in the spring or early summer. Animals should not be allowed to graze until the following year.

7. If land is to be used for hay and/or grazing, the soil should be sludged or injected, followed by discing and fallowing over winter. The crop should be planted in the spring; cutting and/or grazing is then at the discretion of the farmer.

ACKNOWLEDGEMENTS

This project was funded by contracts and grants from the Research Management Divsion (Alberta Environment), Municipal Engineering Branch (Alberta Environment), and the Alberta Environmental Research Trust. The authors are very grateful to these agencies for their support throughout the three years of the project. The assistance of David Spink of the Municipal Engineering Branch contributed greatly to the success of this project. The authors are especially grateful to Brian Hammond for co-ordinating funding and research.

The City of Calgary and the town of Crossfield were very helpful in providing information, samples, and advice. In particular, the authors appreciated the assistance of Bob McIntosh and Wally Meckelborg of the Bonnybrook Sewage Plant. Special thanks are extended to Ray Pierson of the Shepard Sludge Lagoon facility for his patience and co-operation. The authors are also grateful for permission to take samples on the properties of John Cornish and Bob Copithorne. The editorial assistance of Adair Hope and the word processing efforts of Grace Lebel and Trish Harrison are gratefully acknowledged.

Finally, the authors wish to extend their sympathy and thanks to Scruffy, the horse who unwittingly provided many parasite samples for methods development in the Kananaskis Laboratory.

1. INTRODUCTION

Municipalities, regardless of size or industrial complexity, are faced with the problem of sewage treatment and the ultimate removal of the final products, effluent and sludge. Although disposal can be complicated by statutory regulations and public opinion, the plant nutritive and soil conditioning value of the final products must be recognized. Sewage by-products are disposed of either on land or to watercourses. The latter choice is commonly used for liquid effluent, and the thicker sludge (about 11% solids) is spread on land, often agricultural fields as is done at Calgary. Other methods of terrestrial disposal, such as burial, are used but, in order to reduce costs and to gain the fertilizer value from sludge, it is preferable to spread this material on agricultural fields. In dry areas, liquid effluent may be sprayed onto land and may indeed be an important economic resource. Despite the agricultural and economic advantages of terrestrial disposal, sludging or spray irrigation of effluent carry with them a variety of short- and long- term hazards of which disease potential for both man and his animals is a major part.

This project, which has been of three years duration, has addressed the potential health hazards of the application of sewage end products upon farmlands. The investigation proceeded on a yearly basis in a sequential fashion so that groundwork was laid for each succeeding step. The projects undertaken and the timing of such endeavors are outlined below and include those tasks which were designated and, in addition, other objectives not specifically spelled out but which were deemed necessary or complementary to any particular aspect.

In the initial year, a literature review was carried out followed by a determination of parasite stages, indicator bacteria, and *Salmonella* from the following sources: (1) the Crossfield study involving investigations of the lagoon sewage disposal system of that town plus examination of a barnyard, a slightly grazed pasture, and an ungrazed hayfield which had been spray irrigated the year before with effluent from the 12-month lagoon of the disposal system; and (2) the Bonnybrook study which dealt with raw sewage, the anaerobic digesters and effluent at the main plant, and the sludge lagoons and sludged fields at Shepard which were the recipients of materials from the anaerobic digesters. In addition, work was conducted on the culture of parasitic protozoans, viability studies of parasite stages, and methodology with reference to the best means of determining the presence of parasite stages from non-clinical samples. During the latter portion of the first year, it was decided at a workshop that statistically reliable determinations of the various organisms be made from sludged soil and that the vertical distribution of parasites be ascertained from the same fields.

The second year of the study involved a continuation of statistically valid sampling methods used on fields sludged from 1978 to 1980 on a seasonal (quarterly) basis. Examinations of materials from a slaughterhouse were made to determine the organisms contributing to sludge from that source and both egg and infective larval analyses were commenced on the fields. Investigations were accomplished (1) on the fate of parasite stages in sludge, sludged soil, and faeces; (2) the viability and contribution of free-living nematodes; and (3) a beginning of the quantification of numbers of larval worms (as opposed to eggs) from sludge and sludged soils. With the co-operation of Dr. Laila Sekla, of Cadham Provincial Laboratories, Winnipeg, viral analyses were begun while a literature review was conducted at Kananaskis. A field sludged and disced but not planted in 1981 revealed few if any parasite stages after a period of exposure to the elements and led to steps being taken to prepare for the third and final year of work. These steps involved adaptation of the methodology for the detection and identification of certain pathogenic bacteria and renewed efforts on the isolation of parasite larval stages.

The terminal year of the investigations was concerned with the fate of parasite stages on (1) the 1981 fallow field; (2) a field sludged but neither disced nor planted in 1982; (3) a pasture; (4) a control field; and (5) buried sludge. Certain primary and secondary bacterial pathogens were likewise determined from the above sources and a method was found to quantify a broad group of pathogens and allergens.

Prior to the third year of the project, it became evident that proving a health hazard was an insurmountable task. Instead, another approach was chosen, the thrust of which was not to demonstrate that a potential health hazard existed but, rather, to show that the potential for infection was not present.

The prime effort of the project, then, was to determine when the sludged fields could be considered safe. The model for safety was the background levels defined by the conditions of a control field, specifically when the sludged fields approximated the control field with regard to:

1. the numbers of parasite stages;
2. the numbers of total coliforms, i.e. Escherichia coli, Enterobacter aerogenes, and Citrobacter freundii;
3. presence or absence of viruses;
4. presence or absence of faecal coliforms;
5. the presence or absence of primary pathogens, i.e. Salmonella, Staphylococcus, and Mycobacterium;
6. the presence of secondary pathogens which could be assigned to the Enterobacteriaceae. This phase would be divided into two parts:
   (a) those genera of the above noted family which have been noted previously as being secondary pathogens; and,
   (b) the total numbers of Enterobacteriaceae as related to allergen capability;
7. the presence or absence of Pseudomonas spp.

Because we were informed that in the future it was conceivable that reasonably fresh, as opposed to the aged sludge presently used, would be applied to fields, parasite and microbial analyses were again conducted upon digester sludge from Bonnybrook. Likewise, analyses were made upon Fish Creek plant material (raw sludge and 38°C digester sludge).

At the end of the third year, a symposium involving active workers in the field of health hazards of sludge was held at the Kananaskis Centre and the results of the meeting were published in book form[1]. Additionally, prior to the symposium, an open meeting was held so that the results could be disseminated to interested people and so that they could question the participants of the symposium on various aspects of the work.

---

[1] Wallis, P.M. and D.L. Lehmann. In press. Biological health risks of sludge disposal to land in cold climates. U. Calgary Press.

2. LITERATURE SEARCH: PARASITOLOGY

Human parasites, which may be found in Alberta and which possess an intimate relationship with sewage,are noted in Laird et al. (1980) and are quite similar to those referred to in the recent studies in British Columbia (Parsons et al. 1975) and Ontario (Graham 1981).

In Ontario, the Ministry of Health (Scholten and Yang 1975) indicates that the protozoans, Entamoeba histolytica and Giardia lamblia, are the most common pathogens encountered while Balantidium coli is infrequent. Flukes are not mentioned in the report, but the beef tapeworm, Taenia saginata, and the dwarf tapeworm, Hymenolepis nana, have been recorded, though the eggs of the latter occurred with such infrequency as to be negligible in sewage samples. Taenia saginata, on the other hand, could pose a larger problem even though the recorded rate of human infection was not substantially greater than that of Hymenolepis (35 vs 20). Whereas the eggs of Hymenolepis are found individually in the faeces, eggs of Taenia are clumped together and the entire gravid proglottid containing tens of thousands of eggs may be released into the sewage system. Faecal examinations (which are employed to obtain such public health data) could greatly underestimate Taenia saginata infection unless the presented specimen happens to contain a proglottid.

Roundworms were the most common in Ontario, with Ascaris being the most numerous, followed by the whipworm, Trichuris trichiura, the hookworms, Strongyloides, and some roundworms of cats and dogs (Toxocara spp.). The pinworm, Enterobius vermicularis, is certainly present and infection rates of 60% among children have been recorded from Canada (Chandler and Read 1961), but the high rate of infection does not reflect a similar number of eggs in sewage because the life cycle of the worm involves deposition of the eggs on the perianal area and not commonly in faecal material. In British Columbia, Parsons et al. (1975) quote Bowmer (1973) regarding the types and prevalence of animal parasites, of which the pathogens noted are Entamoeba histolytica and Giardia lamblia (Protozoa), the tapeworm, Taenia, and the roundworms, Ascaris, Trichuris, the hookworms, and Enterobius. In Alberta (Laird et al. 1980), Giardia and Entamoeba are reported to be the most common enteric protozoans, while Ascaris, Enterobius, *Trichuris*, *Taenia*, and *Diphyllobothrium* are the most numerous indigenous worms.

2.1 INFLUENCE OF SEWAGE TREATMENT ON PARASITES

As will be noted in the following discussion, the various sewage treatment processes do not eradicate all of the animal parasites initially present in the raw sewage and the rate of destruction of all species of parasites is not similar (Kabler 1959). *Ascaris* spp. are the most resistant and they also are considered to be the key organism to authenticate the effect of treatment (Krige 1964; Fitzgerald 1979).

2.1.1 Primary Treatment

Cysts of *Entamoeba histolytica* were not removed during a three hour settling period (Cram 1943) but Wang and Dunlop (1954) reported a 48% reduction of cysts (from 52 to 27/L) during the settling period. They also noted that chlorination caused a slight further decrease to 24 cysts/L. Additionally, they showed that 46% of *Entamoeba coli* can survive primary treatment and chlorination.

Fifty-one percent of the eggs of *Taenia saginata* settled in an 38 cm column in 15 minutes while, after 2 hours, 98% had deposited (Newton et al. 1949). Russian research (as reported by Greenberg and Dean 1958), indicated that 97% of all worm eggs from influent sewage (including those of *T. saginata*), were removed in an Imhoff tank. Newton et al. (1949) stated that with a settling period of 2 hours and with an overflow of 700 gallons/ft^{2}day (33 845 L/m^2/day) that a majority of *Taenia* eggs would be deposited during primary treatment/sedimentation, but there would still be a significant carryover in the effluent. Liebmann (1964) states that eggs of *T. saginata* are among the slowest of all worm eggs to settle (0.75 to 1.0 m/day) and could be wind dispersed. Bhaskarian et al. (1956) studied the effect of 2.5 hour period of sedimentation upon nematode eggs; they found that 67-75% of *Ascaris* eggs, 46-70% of hookworm eggs, and 52-60% of *Trichuris* eggs settled out during that time interval. Wang and Dunlop (1954) dealt with the elimination of *Ascaris* eggs during primary sedimentation which was followed by chlorination and found an 80% diminuation, but failed to indicate the settling time. In unchlorinated samples, the reduction in numbers was 54% of the 30 eggs/L of raw sewage.

2.1.2 Sludge Systems

Cram (1943) states that *Entamoeba histolytica* is not influenced by activated sludge and then goes on to say that the parasite will survive 10 days at 30°C. Fox and Fitzgerald (1976), however, report that hundreds of *E. histolytica* were found in anaerobically digested sludge although none were viable.

Kott and Kott (1967) recorded 4 cysts/L of *E. histolytica* in raw sewage and only 1 cyst/L in the final effluent. Fox and Fitzgerald (1978) found from 90 to 530 *Giardia lamblia* cysts/L in raw sewage and none in anaerobically digested sludge. They mentioned that flotation concentration was of no value for detecting cysts and that stained, wet mounts were necessary.

Coccidian oocysts in anaerobically digested sludge became non-viable at 39°C (Jolley and Fitzgerald 1976), and Fitzgerald and Ashley (1977) found that *Eimeria stiedae* survived 21 days at 22°C and then sporulated normally when placed in 2% potassium dichromate. These oocysts were rendered non-viable in 4 to 5 days at 28°C. Owen and Crewe (1979) noted that there were but a few eggs of *Taenia saginata* to be found in sludge and Graham (1981) suggested that *Taenia* eggs would probably not survive in sludge amended soil more than a few weeks during warm weather but survival could be extended to a month or more if sludge is applied during cool weather.

Rudolfs et al. (1951b), Keller (1951a), Forstner (1970), Foster and Engelbrecht (1973), and Fitzgerald and Ashley (1977) found that sludge, aerobically or anaerobically digested, contained *Ascaris* eggs, and Hays (1977) reported the pinworm, *Enterobius vermicularis* to be common in sludge. Fox and Fitzgerald (1978) found *Ascaris* eggs and, in addition, *Trichuris*, *Toxocara*, *Enterobius*, *Taenia*, *Hymenolepis*, *Eimeria*, *Isospora*, and *Entamoeba coli*. Grabow and Nupen (1972) reported the presence of the eggs of *Ascaris* (119/L), *Taenia* (87/L), and *Trichuris* (13/L). Alba and Sudo (1964) found that *Ascaris* eggs became concentrated in sludge in that 80 eggs/L were found in raw sewage, whereas 700 eggs/L were found in digested sludge. They also affirmed that *Ascaris* eggs settle rapidly in that they found none in effluent. In an experiment, Fitzgerald and Prakasam (1978) placed *Trichinella spiralis* cysts in an anaerobic digester at 35°C and found a maximum survival rate of 96 hours as determined by animal (rat) feeding.

Cram (1943) notes that hookworm and *Ascaris* eggs were not damaged by activated sludge and that *Ascaris* eggs showed little injury under the influence of anaerobic digestion over a period of 3 months. After 8 months, 10% were still viable. Hookworm eggs survived 41 days at 30°C but larval development was suspended until a more favorable environment was achieved. Kebina and Ploscheva (1974) found that development of *Ascaris* eggs was deterred in anaerobically stabilized sludge because of lack of oxygen, but normal development ensued in air at 20 to 27°C. Fitzgerald and Ashley (1977) introduced pig ascarid eggs (*Ascaris lumbricoides* var. *suum*) to digested sludge for 21 days at 22°C and found that 46 to 96% of the eggs developed upon exposure to air. Twenty-one days at 38°C, however, killed all of the experimentally exposed eggs. Jolley and Fitzgerald (1976) found that *Ascaris* eggs could survive incubation at 39°C. They also found that the eggs of "stomach nematodes" (gastro-intestinal nematodes [GIN]) showed slight reaction to anaerobic digested sludge at 39°C but that the second and third stage larvae succumbed.

In addition to the citations noted above, researchers have stressed the importance of temperature in the survival of cysts and eggs. Liebmann (1964) and Wiley and Westerbrook (1969) stated that sludge must exceed the usual 35°C of anaerobic digestion to be effective in killing parasite eggs. Liebmann (1964) indicated that all parasite eggs were killed in heated sludge over two months; in unheated sludge they survived for 3 months. Keller (1951a and 1951b) stated that *Ascaris* eggs were killed in sludge heated to 53 to 54°C for 24 hours, and Rudolfs et al. (1951c) and Kabler (1959) recommend heating while the Water Pollution Control Federation (1972) specifically states that 60°C for not less than 40 hours will kill all pathogens. Kawata et al. (1977) affirm this position.

Rudolfs et al. (1951a) write that sludge held for two hours at 45°C has no effect upon *Ascaris* eggs while a 30 minute retention at 50°C will initially retard development leading to death in two hours. A 10 minute exposure at 55 to 60°C will kill all eggs. The United States Environmental Protection Agency (1974 and quoting Roedigen) states that, in sludge kept at 50°C, *Entamoeba histolytica* is killed in 5 minutes while 60 minutes at the same temperature kills *Ascaris* eggs.

Composted sludge may reach temperatures of 60°C and, according to Gotaas (1956), this will kill all pathogens. Wiley (1962) and Almasi et al. (1971) concur. To the contrary, Van Kleek (1958) and Goleucke (1972) and Sopper and Kardos (1973) believe that sludge should be further heat treated to effectively destroy pathogens.

2.1.3 Effect of Physico-chemical Treatment on Parasites

The effect of chlorination upon the survival of parasites has been discussed by several authors. Rudolfs et al. (1950) found that normal residual chlorine killed *Entamoeba histolytica* but Kott and Kott (1967) stated that 8 ppm over a period of 6 hours were required to destroy the organism. Wang and Dunlop (1954) reported that aeration of primary sludge sediment followed by chlorination at 3 mg/mL reduced the viability of *E. histolytica* by 54%. Hays (1977) states that the quantity of chlorine customarily employed to treat sewage effluent is not harmful to either protozoan cysts or worm eggs.

Iwanczuk and Dozanska (1956) state that 400 mL/L of chlorine at pH 2 is required to kill *Ascaris* eggs in sewage, and Wang and Dunlop (1968) reported aeration of primary sediment followed by 3 mg/L of chlorine would reduce the viable population of *Ascaris* eggs by 80%. Krishnaswami and Post (1968) further wrote of the resistance of *Ascaris* eggs to chlorination being as high as 100 ppm. Kabler (1959) indicated that eggs were resistant to chlorination.

Cram (1943), Rao (1973), and Van Fleet (1973) mention that chemical coagulants are effective in precipitating parasites, including both protozoan cysts and helminth eggs. It is presumed that such precipitation will remove infective agents from the effluent but will tend to concentrate still viable eggs and cysts in the sludge. Sivinski and Whitfield (1975) show that temperature in combination with radiation is an effective way to destroy pathogens in sludge.

2.1.4 Lagoons

A review of the literature by Rudolfs et al. (1950) revealed that cysts of *Entamoeba histolytica* could remain viable for weeks, and perhaps months, in water (lagoon) and sewage.

Parsons et al. (1975) speculate that cysts, even if they were not removed by sedimentation, would not survive for more than a few weeks in storage. Cram (1943), however, believed that primary effluent is of major importance in the dispersal of Entamoeba cysts. Bryan (1974) believes that sand filtration is the only effective means to remove Entamoeba cysts (and Ascaris eggs).

The eggs of parasitic worms would be removed by settling in sedimentation ponds (Kabler 1959) and this supposition is supported by previously-cited work regarding settling in primary treatment (Newton et al. 1949; Bhaskarian et al. 1956; Greenberg and Dean 1958; Alba and Sudo 1964). Parsons et al. (1975) concur with this assessment, but Cram (1943) feels that it is uncertain that Entamoeba cysts would be settled out. Because the eggs of Taenia saginata settle at 0.75 to 1.0 m/m (Liebmann 1964), as previously mentioned, feels that under appropriate conditions they would be wind dispersed.

2.2 SURVIVAL OF PARASITE STAGES ON FOOD PLANTS AND FODDER

The survival of eggs and cysts in soil and on vegetation after exposure to treated sewage products is of concern to the possible transmission of such parasites to man and animals.

Rudolfs et al. (1950) found Entamoeba histolytica cysts applied to tomatoes and lettuce via sewage products will survive up to one week in temperate climates and two weeks in tropical locations. Doran et al. (1976) stated that E. histolytica cysts survive for three days on plant surface and for eight days in soil.

Rudolfs et al. (1951b) worked with Ascaris eggs and stated that vegetable contamination could exist for one month after placement and that rainfall or normal washing did not remove all of the infective agents. They also stated that lettuce (which has a short period of growth) could carry eggs through the harvest.

Table 1 indicates the periods of time which parasite cysts, eggs, and larvae have been found to remain viable on plants and soil. Soil is included in this tabular presentation because any type of crop planted in sludged soil may acquire pathogens by direct contamination, particularly in the case of root crops.

Table 1. Survival times of various parasites on plants and soil.[a]

Organism	Medium	Survival Time
Ascaris eggs	Soil	2 to 5 years
	Soil	Up to 7 years
	Plants, fruit	1 month
E. histolytica cysts	Soil	8 days
	Tomatoes	8 to 42 hours
	Lettuce	8 hours
Hookworm larvae	Soil	6 weeks
E. histolytica cysts[b]	Soil	6 to 8 days
	Vegetables	1 to 3 days
	Water	8 to 40 days
	Vegetables	3 days
	Water	months
Ascaris eggs	Soil	to 7 years
	Vegetables, fruit	27 to 35 days
	Irrigated soil	2 to 3 years
	Soil	6 years
Hookworm larvae	Soil	6 weeks
Liver Fluke eggs	Dry hay	a few weeks
	Improperly dried hay	over a year

---

[a] Taken from Sepp (1971); Bryan (1974); Dunlop (1968; *In* Parsons et al. 1975).

[b] Taken from Carroll et al. (1975).

2.3 SOURCES AND PRESENCE OF PARASITES ON SLUDGED FIELDS

The bulk of genuine parasite eggs encountered have been those of domestic animals. This finding affirms the work of Liebmann (1964) who found 90% of eggs from sewage of Central European cities are of animal origin. Riemers et al. (1979) and Prestwood (1980) state that slaughterhouses constitute an important source of animal pathogens to sewage and, ultimately, to sludged fields. The World Health Organization (1967) states that soil samples contain a wide range of the eggs of worms of domestic and wild animals,and Riemers et al. (1979) and Prestwood (1980) indicate that wild animals living on sludged fields can act as reservoirs of pathogens. Presumably such species can also deposit their own parasite stages on the fields.

Although some of the "genuine" parasite eggs (i.e., those fitting published dimensions or having unique morphologies) found in sludged land can be attributed to animal parasites, many of the eggs seen and recorded could conceivably be those of free-living nematodes which resemble those of parasites. The World Health Organization (1967) refers to this problem in mentioning that in soil, in addition to parasite eggs, the eggs of numerous types of free-living animals are present in considerable numbers and Levine (1968), with particular reference to strongyle eggs, states that attempts can be made to identify nematode eggs from recorded measurements "but it is extremely difficult if not impossible as a practical matter."

2.4 DISTRIBUTION OF PARASITE EGGS IN SOIL

The placement of parasite eggs on soil departs from the random distribution which might be expected and, instead, local accumulations or pockets of eggs have been encountered. Graham (1981) found that sludge applied to experimental plots could, because of irregularities in the surface, be spread unevenly on the soil surface. This results in thicker patches of sludge which might be expected to contain more eggs. He also made the following observations:

1. If there was a heavy rain before the sludge dried, the sludge would become more evenly distributed.
2. If the soil was dry upon application, the moisture from the sludge would quickly wet the underlying soil

and there would be little lateral movement on level ground.

3. If the soil surface was already saturated before application, the sludge would flow rapidly into surface depressions, resulting in an uneven distribution.

Graham (1981) also noted that sludge is thickened by settling in lagoons which could result in worm eggs having an uneven distribution.

Beaver (1975) found that soil particles can be moved some distance by heavy rainfall and, in addition, found that rain on unplanted soil can result in the concentration of nematode eggs under a layer of top soil. The World Health Organization (1967) found that, because of the sorting action of wind and rain on soil particles, Ascaris eggs are not randomly distributed. All of these mechanisms support the "pocketting" observation.

2.5 PARASITE STAGES ON LAND

Muller (1953) reported that Ascaris eggs can survive in soil for 5 to 7 years while Soulsby (1968) states that the eggs are very resistant to adverse conditions (drying, freezing, chemicals) and can remain alive for five or more years. Hot and dry conditions such as found in sandy soil exposed to sunlight, however, can limit their survival to a few weeks. Georgi (1969) indicates that the eggs of Toxocara, like those of all ascarids, are resistant to environmental conditions, particularly when located in poorly drained clay and silt soils. Forstner (1970) states that Ascaris eggs can withstand repeated freezing-thawing and some degree of heating. Between 30 to 45°C, development is slowed but not impaired whereas exposure to 50°C for 10 minutes reduced viability by 10%. All were killed by 15 minutes of exposure to 50°C. The World Health Organization (1967) states that, experimentally, Ascaris eggs are killed in eight days at 38°C, and, in soil, a temperature of 40°C is lethal. Additionally, Ascaris eggs are destroyed in the soil when the moisture content is 4% or less. Surface temperatures in bare soil can be quite high when exposed to strong sunlight. Graham (1981), in Ontario, found that surface temperatures could be as high as 47°C on the surface but not over 26°C at a depth of 8 cm. Levine (1968) found even higher temperatures (60°C) in

Illinois soils and equally high temperatures in exposed sheep pellets. If vegetation cover is present, soils would be partially protected and moisture content would be higher. Beck (1979) writes that *Toxocara* eggs can readily withstand winter while Krasnonos (1978) reports a maximum survival time of *Ascaris* eggs in soil as being 15 years in Russia. Regarding another massively shelled worm egg, Levine (1968) records that *Trichuris* may survive for several years in soil under ideal conditions and that survival is correlated with such environmental influences as soil type, moisture, temperature, and sunlight.

Concerning survival of strongyle eggs in soil, Soulsby (1968) states that the eggs of *Strongyloides* (except *S. stercoralis* where larvae are usually deposited) from domestic animals have some degree of resistance depending upon moisture, temperature, and enclosing matrix. The infective larvae, however, cannot resist dessication because they lack an enclosing sheath.

Soulsby (1968) discusses the resistance of the eggs of *Strongylus*, *Triodontophorus*, *Chabertia*, *Oesophagodontus*, *Trichonema*, *Poteriostomum*, *Gyalocephalus*, and *Oesophagostomum* in soil. Development of the larval stages is inhibited by lack of moisture and a temperature of less than 7.2°C. He also states that, if the temperature reaches 9°C, development will proceed and hatching will occur. Eggs which have not embryonated have difficulty in surviving temperatures below freezing, but those in the pre-hatch stages are capable of survival at low temperatures for several weeks. Eggs which have not developed to the pre-hatch stage are destroyed by drying while those eggs which have reached the pre-hatch stage can survive dessication for several weeks in a dormant state. The return of moist conditions (and the presence of oxygen) will result in hatching.

Trichostrongylus and *Haemonchus* tend to be similar in their behaviour in soil (Levine 1968; Soulsby 1965). Both show little or no embryonic development at a temperature of less than 9°C and those eggs containing larvae are more resistant to freezing and dessication than are those in early development. The infective larvae can remain viable for in excess of six months regardless of conditions. Soulsby (1968) in a later communication states that *Trichostrongylus* is more resistant than *Haemonchus* but that both species are capable of overwintering. Kates (1950) found that the infective larvae of *Haemonchus contortus* are not particularly resistant to adverse conditions and "regardless of the conditions of exposure it is unlikely that the larvae will survive for more than 6 months and in fact the majority will have been destroyed much earlier than this," and that few larvae survive over the winter.

Drurie (1961) reported that infective larvae of *Haemonchus*, *Osteragia*, *Trichostrongylus*, *Cooperia*, and *Oesophagostomum* survive in faecal pads during dry weather for 4 months or longer and, with the advent of moisture, the larvae leave the faecal pad and contaminate the surrounding area. Georgi (1969) reports that certain species of gastro-intestinal nematode eggs/larvae are well adapted for survival in cold climates including *Trichostrongylus*, *Haemonchus*, *Cooperia*, *Chabertia*, *Oesophagostomum*, *Osteragia*, and *Nematodirus*.

In addition to those physical features detailed above, certain other deterrents to parasite longevity have been reported. A report of the World Health Organization (1967) suggests that the low rate of survival of *Ascaris* eggs in lower levels of soil may be due to the predatory activities of mites and fungi. Jones et al. (1979) found that some 30% of *Ascaris* and *Taenia* eggs contained in sludge show degenerative changes due to fungal attachment, while those eggs which have been ingested by earthworms and subsequently re-deposited in a casting show but a 3% fungal attack rate.

Regarding the longevity and accumulation of eggs in contaminated soil, it is conceivable that a number of physical and biological factors play a role. The parasite load is modified according to the vagaries of these factors. For example, Spindler (1929) reported that, with continuous faecal application, there was no appreciable buildup of infective eggs indicating that eggs do not survive for years. He theorized that eggs probably do not overwinter. Jolley and Fitzgerald (1976), to the contrary, believe that, as application of sludge (containing parasite stages) continues, the accumulation of parasite stages becomes greater.

2.5.1 Infectivity of Parasites

Fitzgerald (1981) has reported the only authenticated instance of the transmission of a parasite to an animal host from sludge treated soil naturally containing ascarid eggs. Forty-three of 134 pathogen-free pigs exposed to both land applied sludge and to dried sludge (Nu-Earth) buried to a depth of 15.3 cm were eventually found to be parasitized with Ascaris lumbricoides suum. The eggs in these sludges remained unembryonated until exposed to air. Fitzgerald (1981) failed to transmit the human ascarid, A. lumbricoides, to hogs. Earlier, Jolley and Fitzgerald (1976) reported an experiment where cattle were pastured on land upon which digested sewage sludge had been repeatedly applied for two years. In this two year period, there was no appreciable difference of parasite burden (coccidia and helminths) between the experimental and the control populations. The authors conclude that after two years there was no detectable build-up of parasites, but, as the application level increases, the accumulation of parasites becomes greater. Because the effects of sludge on parasites are relatively unknown, further monitoring was recommended.

Wood et al. (1975) made a survey of rabbit parasites (coccidia) on land which had been irrigated with chlorinated effluent and found that in young rabbits the parasite load was substantially greater than in animals from control sites. In mature rabbits, the situation was reversed. No mention was made in the report of isolating coccidian parasites in the effluent. Instead the experiment was concerned with the survival of oocysts from the infected hosts upon the irrigated land, the conclusion being that survival of oocysts was greater on irrigated land, presumably because of the increased amount of vegetation, high humidity, relatively warm temperature and a good supply of oxygen.

Other assessments of the problem are speculative in nature and run the course of incipient danger to no concern. Morrison and Martin (1976) state that there is a significant lack of reliable data on natural transmission of parasites. Fitzgerald (1976) indicates that most reports discuss survival of parasites under various conditions but fail to mention actual instances of disease transmission. Fox and Fitzgerald (1978) say that further information is needed to see if ascarids and tapeworms in municipal wastes pose a threat to human or animal health, and Fitzgerald (1976) suggests that the use of sludge may disseminate parasites to the environment with possible subsequent transmission to animals and man. He further states that the area of natural transmission has been inadequately investigated. ECO/LOG (1978) mentions that the survival of parasites in sludge constitutes a potential health hazard which must be taken into account in the formulation of guidelines for sludge usage.

Laird et al. (1980), in their document on human intestinal parasites in Canada, make the following comments: "we remain in ignorance of the capacity of the treatment procedures to destroy infective or resting stages of human intestinal parasites, whether protozoan cysts or helminth eggs and larvae. Again, sludge derived from such systems is sometimes used as a fertilizer--despite our lack of knowledge of whether parasites capable of harming man can or cannot be disseminated in this way."

Further, Liebmann (1964) believes that partially digested sludge could spread taeniasis, and Logan and Miller et al. (1979) feel that sludge should not be used on vegetable crops because of the potential for the transmission of pathogens. Miller et al. (1979) specify that lactating dairy cattle should not be exposed to pasture or hay previously exposed to sludge because of the possibility of udder contamination resulting in worm eggs reaching the milk. They also feel that, even with low numbers of parasites, there should be a restriction on the use of sludge for vegetable crops. Oldham (1980) states that adequate precautions should be taken to minimize public contact with fresh or even secondary treated sewage. Parsons (1975) says that evidence for transmission of parasites from secondary effluent is sparse, but that pathogens do survive secondary treatment and it follows that such organisms can contaminate food crops to the extent that disease symptoms can occur in humans. Carroll et al. (1975) say that pathogens survive sludge digestion and that the potential exists for human and animal infection.

Fitzgerald and Mansfield (1970) mention that there is no direct evidence concerning calf infection related to exposure to sludge containing eggs of ascarid worms.

There are reports which indicate that the problem may be minimal. Ewing and Dick (1970) write that no incidence of disease transmission had been traced to sludge disposal, and Sabey et al. (1973) believe that digested sludge poses few, if any, health hazards. Obviously, then, the problem of parasite transmission is complex. In this regard, Levine (1968) makes the following statement: "Actually, our knowledge is quite general, and when attempts have been made to study the problem more specifically, we have become entangled in such a meshwork of multiple interfacing variables that our results have been inconsistent. The point has not yet been reached where reliable predictions about the potential transmissibility of a particular parasite at a particular time and place are possible."

3. LITERATURE SEARCH: THE BACTERIA

The following review is divided into two sections. The first is an overview, somewhat cursory in nature, providing a general background on the nature of those bacteria of pathogenic or indicator significance that might be encountered during a search of sewage disposal facilities. The second portion of this review is more comprehensive and is concerned with pathogen survival on sludged fields.

3.1 BACTERIA FROM SEWAGE AND ITS BY-PRODUCTS: AN OVERVIEW

Parsons et al. (1975) state that few bacterial disease entities were reported from sewage in British Columbia but that *Salmonella* was the most common, followed by *Shigella* species. Jensen (1954) found *Mycobacterium tuberculosis* in sludge derived, in part, from tuberculosis sanatoria and noted that they were able to survive for 11 to 15 months on drying beds. Dudley et al. (1980) examined sludge and found *Staphylococcus*, *Klebsiella*, *Salmonella*, *Pseudomonas*, *Mycobacterium* spp. as well as other gram negative enteric bacteria and indicator organisms. Johnson et al. (1977) noted that various organisms could also be found in sewage and effluent, including gamma haemolytic streptococci, micrococci, *Enterobacter*, *Escherichia*, *Citrobacter* and certain diptheroids. Kenner et al. (1971) isolated 23 O-serotypes of *Salmonella* from digested sludge, and Taylor (1966) stated that most of the serotypes of *Salmonella* found in man are also found in the "lower" animals with the exception of *S. typhi* and the paratyphoid organisms which are restricted to humans.

Considerable emphasis has been placed upon the effect of sludge on bacteria. Parsons et al. (1975) said that in excess of 90% of pathogenic bacteria were removed in activated sludge, and Bryan (1974) found that activated sludge treatment followed by secondary sedimentation removed 90% of coliform and pathogenic forms remaining after primary sedimentation. Kabler (1959) reported that the activated sludging process reduces bacterial counts of treated effluents from 88 to 99% with the coliforms and enteric pathogens (*Salmonella* and *Shigella*) being destroyed at a slightly higher rate (91 to 99%). Those organisms producing tuberculosis (*Mycobacterium* spp.) die at a lesser rate of 88%. Gritzuk (1973) published data to the effect that anaerobic digestion reduces the numbers of pathogenic bacteria by 99.8% and Kenner et al. (1971) recorded a slightly lower figure (97%) for faecal coliforms, but the survivors still amounted to 390 000/100 mL.

Dedie (1955) wrote that *Salmonella* survive in raw sludge for 180 days and remained viable for 45 days in digested sludge. Harbourne (1978) stated that the salmonellae are capable of surviving all forms of sewage treatment, including that of activated sludge. Sepp (1971) reported that *Salmonella* and *Shigella* are often found in sewage and in sludge and that they are resistant to environmental conditions, although their resistance is less than that of the coliform bacteria. In contradiction to the above, Sepp (1971) and Krone (1968) suggested that *Salmonella* is able to survive longer in soil and on vegetation than are the indicator coliforms. Regarding the types of *Salmonella* to be found in sludge, Kenner et al. (1971) were able to isolate 23 serotypes of the organism.

Various authors have reported on the effect of lagoon/sludge stabilization pond environments upon pathogenic microorganisms and indicators. Parsons et al. (1975) said that 90% of such bacteria were removed after several months of incubation. Fitzgerald and Rholich (cited by Kabler 1959) found that lagooning reduced the bacterial population to less than 1% of the original number with *Escherichia coli* dropping from 100 000/mL to less than 100/mL and *Salmonella typhi* was completely destroyed, dropping from 41/mL to none. Kabler (1959) found a reduction of 90% of coliform MPN in more than 50% of the instances in lagoon systems and noted that there was no appreciable difference in amount of reduction during various seasons of the year. Kabler (1959) felt that the fate of all enteric bacteria would be similar to that of the coliforms as the observation had frequently been made that coliforms and enteric pathogens tend to have survival rates of the same magnitude. Cody and Tischer (1973) recorded a 97% reduction of coliforms during 15-21 days in a stabilization pond. They found very few *Salmonella* and no *Shigella*. Kott (1973) found that oxidation ponds were quite efficient in removing *Salmonella*. Whereas the organisms were found in the influent sewage, none were detected in the effluent, similar to the results of Lehmann et al. (1981). Bell (1976), working at Taber, Alberta, found 13

Salmonella/100 mL by MPN methods and felt that such small numbers would pose little threat to livestock eating forage irrigated with the effluent. He also found that the Salmonella population was 0.1% of the indicator faecal coliform count.

Parsons et al. (1975) noted that 95 to 99% of bacteria were removed from secondary effluent if a residue of 1 mL/L of chlorine was maintained for one hour. Dunlop (1968) stated that primary treatment followed by chlorination destroys 99% of the coliforms and enterococci in the effluent. Kabler (1959) stated that the efficiency of chlorination is dependent upon the amount and nature of the organic material, the amount of ammonia, pH, temperature, the chlorine dosage, and the type of test used to determine the residual chlorine. It was his contention that a good reduction (96 to 99%) of coliforms, typhoid organisms, and tuberculosis bacteria could be achieved with sufficient contact time and residual chlorine. Sivinski and Whitfield (1975) found thermo-radiation of sludge to be quite efficient.

The viability of bacteria in the air, in soil, and on plants has received much attention because of the possibility or potential of infection from those sources. Table 2 incorporates data taken from Sepp (1971) and Parsons et al. (1975). Oldham (1973), dealing with effluent irrigation and alfalfa, found that 90% of coliforms and 99% of faecal coliforms died within one week. He also reported that sewage coliforms could survive, and even multiply, on alfalfa with a sufficiently high moisture content, but that they would die in a few weeks on drying hay. Norman and Kabler (1954) recorded that vegetables irrigated with water high in coliforms obviously have greater counts than those treated with purer water, and that there tend to be more coliforms on leafy, as opposed to smooth, vegetables. Sepp (1971) noted that pathogens survive longer in soil than on plants because soil protects the organisms from radiation, dessication, and extremes in temperature. He recorded that few pathogenic micro-organisms live longer than one, and rarely two, months on plants.

Schmauder and Unger (1960) reported that, if meadow grass is sprayed with sewage during the rainy season, there is a tremendous initial increase in microbial numbers in soil but that within five days about two-thirds die off. In 28 days, the number of coliforms is less than that found on unpolluted grass.

Table 2. Survival times of various bacteria on soil, plants, and in water.

Organism	Surface	Survival Time
Coliforms	Soil surface	38 days
	Vegetables	35 days
	Tomatoes	35 days
	Grass/clover	6 to 34 days
	Grass	14 days
	Clover	14 days
	Clover, 40 to 60% humidity	6 days
	Lucerne	34 days
	Grass to 17°C	46 to 73 days
Salmonella typhi	Soil	1 to 120 days
	Soil	29 to 58 days
	Soil	60 to 70 days
	Vegetables/fruit	1 to 68 days
	Water	7 to 30 days
	Water with humus	87 to 104 days
Streptococcus	Soil	35 to 63 days
	Soil surface	38 days
Faecal streptococci	Soil	26 to 77 days
Shigella	Grass	42 days
	Vegetables	2 to 10 days
	Vegetables	7 days
	Water with humus	160 days

continued ...

Table 2. Concluded.

Organism	Surface	Survival Time
Salmonella	Soil surface	15 to 46 days
	Soil	15 to 280 days
	Soil surface	46 days
	Soil, lower layers	70 days
	Sand soil, sterilized	24 weeks
	Sand soil, sterilized	5 to 12 weeks
	Soil surface potatoes	40 days
	Grass	42 days
	Grass	over winter
Salmonella	Grass/clover	12 to 42 days over winter
	Clover	12 days
	Vegetables	28 days
	Vegetables	7 days
	Vegetables	40 days
	Beet leaves	21 days
	Carrots	10 days
	Cabbage/gooseberries	5 days
	Vegetables/fruits	3 to 49 days
	Dried digested sludge	17 weeks

Dense vegetation is associated with longer survival of coliforms than sparser growth. Kruse (1962) and Bringmann and Trolldenier (1960) found that all clover leaves tested displayed coliforms after spraying with effluent and in five days there was a 50% reduction of numbers. Numbers declined until only 8% of the original population remained after 14 days. They discovered that, instead of reducing the population, rainfall caused increases, presumably because of splash contamination. They found that Salmonella had a maximum survival time of 12 days on clover. Bringmann and Kuhn (1959) stated that when clover was sprayed with sewage organisms, it required 12 to 14 days to reach the control level and that thereafter the numbers remained constant. Novak (1956) found that natural hay drying did not remove coliforms and that there were few differences in coliform counts between dry and fodder plants. He stated that coliforms survive longer than 34 days on alfalfa, and at a temperature of $-18^{o}C$ the time of longevity was 73 days.

Kenner et al. (1971) examined normal soil and found no pathogens or indicator species. After treatment with anaerobically digested sludge, faecal coliforms remained viable for 18 weeks, Pseudomonas aeruginosa for 16 weeks, and Salmonella spp. for 10 weeks. On established grasslands as opposed to relatively clear soil, Salmonella and Pseudomonas lived for as long as 21 weeks after sludge application.

Van Donsel et al. (1967) described a 90% reduction of faecal coliforms on plants in 3.3 days during the summer and 13.4 days in the fall. In the summer, faecal streptococci survived for 2.7 days and 20.1 days in the fall. Boyd et al. (1969) found that there was a 99.9% reduction of E. coli in moist soil in 12 days, while Streptococcus faecalis survived for 22 days under the same conditions. Hinsley et al. (1972) observed that E. coli showed an initial rapid growth in soil containing 5% moisture but by 30 days the total number had decreased 99%.

As previously mentioned, Bell (1976) believed that two days of sunshine would provide sufficient protection for grazing animals on effluent sprayed fields. This position is seconded by Alberta Environment (1977) with the further stipulation that safety from Salmonella infection would also be assured. Jack (1969) and Jannock (1971) reveal that Salmonella will survive longer than two days in sunshine but believe that little threat is posed to grazing animals.

Rudolfs et al. (1951a, 1951b) report no differences in coliform counts on tomatoes which had been irrigated with effluent or purer water, and that the residual coliform count of sprayed tomatoes became equal to that of uncontaminated fruits in five days. They also indicated that rainfall was not a significant cause of splash contamination and that sunlight reduces the numbers of organisms. Rudolfs noted that microorganisms cannot penetrate intact vegetables but will invade broken surfaces.

Muller (1955) found that 69 of 204 grass samples sprayed with raw sewage contained typhoid and paratyphoid organisms, and that three weeks later 5% of the sample was still infected. Kruse (1962) found *Salmonella* 12 days after spraying crops with effluent and Popp (1960) discovered that *Salmonella* survived up to four weeks after effluent spraying of vegetation. Rabinowitz (1954) reported that *Salmonella* survives 46 days on soil surfaces and 70 days in lower layers during the winter. In summer, *Salmonella* disappears from the surface in 15 days while *Streptococcus faecalis* lives for 38 days. Piening (1956) says that *Salmonella* on grass can survive the winter.

The survival of bacterial pathogens in aerosols, whether they be artificial or natural, is a factor of interest because of the possibility of airborne infection. Napolitano and Rowe (1966) reported that activated sludge plants emit up to 10 times as many coliforms as do high rate trickling filters. Sepp (1971) gave figures on aerosol dispersal under the influence of various wind speeds and found that with an 11 kph wind the spray radius would be 70 to 80 m; with an 18 kph wind the radius would be 300 to 400 m. He noted that coliforms could be detected in the aerosol droplets but that they did not occur outside of the radius of spray. Reed (1972) reports that spraying chlorinated effluent yields 1.5 times as many biological aerosols as trickling filters but further states that rapid dessication in flight kills bacteria in the aerosol. He found that death is controlled by relative humidity, temperature and sunlight. Bausum et al. (1976) cites that total numbers of aerosol-borne bacteria in air were 1630/m^3 at points nearest the source and, of the total, only 0.5% were coliform and half of that number were *Klebsiella*. Poon (1966) writes that *E. coli* has a very short longevity in aerosols and, as this organism is a commonly used indicator, studies with it may not accurately reflect the total pathogen load. Sorber et al. (1972) concur with the above and believe that studies should be made to find a new indicator organism (at least for aerosol studies), suggesting that Klebsiella might be suitable because of its fairly resistant capsule.

The possibility that indicator organisms can predict the presence of enteric pathogens is important but not straightforward. Norman and Kabler (1954) state that for every Salmonella there is estimated to be 4800 faecal streptococci and 255 000 faecal coliforms. McGauhey and Krone (1967) report the following numbers of indicators on soil but fail to specify the abundance of Salmonella: coliform, median 1.1 × 10^6; faecal coliforms, median 210 000; and faecal streptococci, median 45 000. These data are comparable to those noted by Norman and Kabler (1954) and it may be presumed that one Salmonella would be 0.1% of faecal coliforms.

Sepp (1971) and Krone (1968) report that Salmonella survives longer than E. coli on vegetation and in soil. Kenner et al. (1971) showed that Salmonella (and Pseudomonas) are more resistant than either E. coli or faecal streptococci. Findlay (1973) states that Salmonella, instead of decreasing in numbers in effluent, can actually multiply. Hess and Breer (1975) found Salmonella live up to 72 weeks in land applied sludge and that aerobic or anaerobic digestion did not significantly reduce the numbers of Salmonella.

The question of the risk of infection amongst animals exposed to the products of sewage treatment is to a great extent unanswered with advocates of both sides presenting their opinions based, in the main, on epidemiological evidence or conjecture. There are, however, two studies which add experimental rationale to the chance of infection. Wood et al. (1975) studied the serotypes of E. coli in birds, rabbits and deer in an area which had been spray irrigated. They found that of 16 E. coli O-serotypes, 11 were recovered from the waste water. Glantz and Jacks (1967) showed that wild birds acquired E. coli serotypes similar to those found in non-chlorinated waste water and that chlorination of the waste water modified the situation.

Morrison and Martin (1976) state that much is known about the survival of pathogens in sewage and its components but that there is a significant lack of reliable data on natural transmission. Katzenelson et al. (1976) showed that spray irrigation led to many cases of shigellosis, salmonellosis, typhoid and infectious hepatitis that were two to four times higher than those in control areas. It should be mentioned here that Taylor and Burrows (1971) found than an infective dosage for _Salmonella_ is greater than 10^6 organisms.

Hanks (1967) states that circumstantial and epidemiological evidence supports a definite relationship between disease and solid wastes. However, in developing countries, the instances of transmission of disease from animal wastes, to humans, are reported as low. Kenner et al. (1971) mention that sludge borne pathogens can live for up to 21 weeks in the top 5 cm of sludge treated soil and the use of unsterile sludge may well contribute to uninterrupted human and animal disease cycles. Miller (1973) writes that the survival of pathogens in soils used for effluent and sludge disposal creates a potential health hazard which could limit the success of this method. Parsons et al. (1975) state that spray aerosols could contribute to the dispersal of infectious agents with subsequent infection by inhalation and suggests that protective buffer zones should be placed around such areas. Carroll et al. (1975) write that, since pathogens do survive sludge treatment and can survive for varying lengths of time, a potential health risk does exist.

Opinions to the contrary do exist, however. Stone (1953) feels that there are problems with raw sewage but that there are few if any hazards from a health point of view when using digested sludge. Ewing and Dick (1970) state there have been no cases of disease transmission traced to sludge disposal operations, and Seabrook (1973) mentions that public health officials do not feel that sludge disposal is a problem as long as the site for spreading is firm, easily drained, gravelly soil. Cody and Tischer (1973) do not believe that stabilization ponds constitute a public health hazard.

Parsons et al. (1975) feel that it is improbable that the use of treated sewage effluent in British Columbia will alter the incidence of bacterial diseases since there are only a few sewage related diseases in the province. The Canadian Wildlife Federation (1977) believes that raw sewage would be unimportant as a disease short longevity in aerosols and, as this organism is a commonly used indicator, studies with it may not accurately reflect the total pathogen load. Sorber et al. (1972) concur with the above and believe that studies should be made to find a new indicator organism (at least for aerosol studies), suggesting that Klebsiella might be suitable because of its fairly resistant capsule.

The possibility that indicator organisms can predict the presence of enteric pathogens is important but not straightforward. Norman and Kabler (1954) state that for every Salmonella there is estimated to be 4800 faecal streptococci and 255 000 faecal coliforms. McGauhey and Krone (1967) report the following numbers of indicators on soil but fail to specify the abundance of Salmonella: coliform, median 1.1×10^6; faecal coliforms, median 210 000; and faecal streptococci, median 45 000. These data are comparable to those noted by Norman and Kabler (1954) and it may be presumed that one Salmonella would be 0.1% of faecal coliforms.

Sepp (1971) and Krone (1968) report that Salmonella survives longer than E. coli on vegetation and in soil. Kenner et al. (1971) showed that Salmonella (and Pseudomonas) are more resistant than either E. coli or faecal streptococci. Findlay (1973) states that Salmonella, instead of decreasing in numbers in effluent, can actually multiply. Hess and Breer (1975) found Salmonella live up to 72 weeks in land applied sludge and that aerobic or anaerobic digestion did not significantly reduce the numbers of Salmonella.

The question of the risk of infection amongst animals exposed to the products of sewage treatment is to a great extent unanswered with advocates of both sides presenting their opinions based, in the main, on epidemiological evidence or conjecture. There are, however, two studies which add experimental rationale to the chance of infection. Wood et al. (1975) studied the serotypes of E. coli in birds, rabbits and deer in an area which had been spray irrigated. They found that of 16 E. coli O-serotypes, 11 were recovered from the waste water. Glantz and Jacks (1967) showed that wild birds acquired E. coli serotypes similar to those found in non-chlorinated waste water and that chlorination of the waste water modified the situation.

Morrison and Martin (1976) state that much is known about the survival of pathogens in sewage and its components but that there is a significant lack of reliable data on natural transmission. Katzenelson et al. (1976) showed that spray irrigation led to many cases of shigellosis, salmonellosis, typhoid and infectious hepatitis that were two to four times higher than those in control areas. It should be mentioned here that Taylor and Burrows (1971) found than an infective dosage for Salmonella is greater than 10^6 organisms.

Hanks (1967) states that circumstantial and epidemiological evidence supports a definite relationship between disease and solid wastes. However, in developing countries, the instances of transmission of disease from animal wastes, to humans, are reported as low. Kenner et al. (1971) mention that sludge borne pathogens can live for up to 21 weeks in the top 5 cm of sludge treated soil and the use of unsterile sludge may well contribute to uninterrupted human and animal disease cycles. Miller (1973) writes that the survival of pathogens in soils used for effluent and sludge disposal creates a potential health hazard which could limit the success of this method. Parsons et al. (1975) state that spray aerosols could contribute to the dispersal of infectious agents with subsequent infection by inhalation and suggests that protective buffer zones should be placed around such areas. Carroll et al. (1975) write that, since pathogens do survive sludge treatment and can survive for varying lengths of time, a potential health risk does exist.

Opinions to the contrary do exist, however. Stone (1953) feels that there are problems with raw sewage but that there are few if any hazards from a health point of view when using digested sludge. Ewing and Dick (1970) state there have been no cases of disease transmission traced to sludge disposal operations, and Seabrook (1973) mentions that public health officials do not feel that sludge disposal is a problem as long as the site for spreading is firm, easily drained, gravelly soil. Cody and Tischer (1973) do not believe that stabilization ponds constitute a public health hazard.

Parsons et al. (1975) feel that it is improbable that the use of treated sewage effluent in British Columbia will alter the incidence of bacterial diseases since there are only a few sewage related diseases in the province. The Canadian Wildlife Federation (1977) believes that raw sewage would be unimportant as a disease source for animals or crops inasmuch as both coliforms and faecal streptococci are reduced during spraying. If chlorine is added prior to spraying, the bacterial load becomes half of that normally encountered, further reducing the danger.

3.2 SURVIVAL OF PATHOGENIC BACTERIA ON SOIL AND SLUDGED SOIL

3.2.1 Survival of Indicator Organisms on Soil

With regard to the commonly used indicator organisms, Bell (1976) noted that faecal coliforms would be destroyed by 10 hours of sunlight. On overcast days, however, there was no decrease in numbers. Oldham (1973) found that longevity was associated with moisture. To the contrary, Boyd et al. (1969) reported that there was a 99.9% reduction of *E. coli* in moist soil in 12 days. Hinsley et al. (1972) observed that *E. coli* showed initial growth in soil with 5% moisture but that there was a 99% reduction in 30 days. In their reviews, Parsons et al. (1975) and Sepp (1971) also reported on the longevity of indicator organisms in the soil. They stated that coliforms may exist for 38 days. Hyde (1976) found that both faecal coliforms and streptococci can live for 7 months in row crops on dry land irrigated with a suspension of anaerobically digested sludge. Edmonds (1976), dealing with total coliforms, found that when 10^6 organisms/g were applied in sludge the numbers decreased to 10^3 to 10^4 within three months and remained generally stable throughout the year. Some reduction occurred during the winter months. Faecal coliforms, when added in sludge at 10^5/g, disappeared from analytical view in 267 days but some aftergrowth occurred during the warmer months. This aftergrowth was similar to that found in unsludged soil (i.e. the background levels). Coliforms, however, were isolated during that interval from both a spring and groundwater in the immediate vicinity but the numbers were small as was the incidence of isolation. Miller (1974) found that, when sludge (with a large population of coliforms) was added to soil, there was an initial increase of numbers peaking at one month after which the numbers decreased rapidly with low numbers still being detectable after six months.

With regard to the survival of coliforms in buried sludge, Trout et al. (1975) found an almost complete eradication of faecal coliforms (Escherichia and Klebsiella) after 2.5 months. They found that there were no inherent faecal coliforms in soil but that there were large numbers of total coliforms and that no relationship existed between total coliform counts and the presence of injected sludge. Dudley et al. (1980) reported persistence of coliforms from sludge applied to agricultural land.

The survival times of various pathogens and indicators from soil are summarized in Table 3. These citations suggest that indicator species have defined survival times on land, but Cliver (1980) points out that E. coli is consistently present in sludge of varying ages. Cliver (1980) states that animals (two legged, four legged, winged, and coldblooded) can act as reservoirs of Salmonella, and Prestwood (1980) states that free-living wild animals are common on sludged lands and could act as reservoirs of pathogens. By extrapolation of the findings in these two citations, it can be said that these animals could contribute pathogens and indicators to sludged land when they are active in the warm months in addition to carrying pathogens and indicators away from sludged land. An alternate hypothesis is that organisms are capable of reproduction on soil and that populations decrease to levels below detection in the winter. This has been shown for Salmonella spp. (Gerba et al. 1975) and may also apply to E. coli.

3.2.2 Survival of Salmonella spp. on Soil

Temple et al. (1980) found that, when E. coli and S. typhimurium (10^6/g) were mixed with faeces and buried in the soil, there was an initial increase in the number of each species which was followed by a decline so that after 8 weeks the numbers were reduced to 10^3 to 10^4/g. Parsons et al. (1975) and Sepp (1971) indicate that Salmonella can survive on the soil surface from 1 to 280 days and in sandy soil for as long as 12 weeks. Beard (1938, 1940) recovered Salmonella typhi from loam and peat soils up to 85 days after application but found survival limited to four to seven days in drying sand, while Dudley et al. (1980) reported Salmonella from sludged fields. Salmonella spp. have been reported from 90% of sludges examined by Hess and Breer (1975). They also stated that the organisms were not significantly reduced in numbers by either anaerobic or aerobic digestion and they could live on sludged fields for up to 72 weeks.

Table 3. Survival of pathogenic bacteria on soil.

Organism	Survival Time	Source
E. coli	10 hours in sunlight	Bell (1976)
	12 days	Boyd et al. (1969)
	30 days	Hinsley et al. (1972)
	38 days	Sepp (1971); Parsons et al. (1975)
	7 months	Hyde (1976)
	>267 days	Edmonds (1976)
Salmonella spp.	1 to 280 days	Sepp (1971); Parsons et al. (1975)
	4 to 7 days in drying sand	Beard (1938)
	85 days-loam and peat soils	Beard (1940)
	72 weeks	Hess and Breer (1975)
	7 months	Hyde (1976)
	300 days	Thunegard (1975)
Streptococcus spp.	35 to 63 days	Parsons et al. (1975)
Mycobacterium spp.	<6 months	Sepp (1971)
	5 to 15 months	Greenberg and Kupka (1957)
Clostridium spp.	7 months	Hyde (1976)

Hyde (1976) reports that Salmonella may survive for up to seven months on row crops and on irrigated and dry pasture sprayed with anaerobically digested sludge. Thunegard (1975) applied a slurry of manure to a field which was then planted in barley and found S. typhimurium and other serotypes of Salmonella after 300 days. Robens (1980) stated that, in the use of sludge as an animal feed supplement, the transmission of Salmonella and Mycobacterium is unresolved. Galton et al. (1954) and Wray (1975) reported Salmonella from the wastes and effluents of slaughterhouses which could eventually reach sludge.

3.2.3 Survival of Other Pathogens in Soil or Sludge

Dudley et al. (1980) found Staphylococcus in sludged fields and Parsons et al. (1975) recorded that Streptococcus can survive in soil from 35 to 63 days. In addition, Dudley et al. (1980), Kenner et al. (1971), and Guentzel (1977) found fluorescent Pseudomonas in sludge but not in soil. Sepp (1971) states that Mycobacterium can live in soil for periods in excess of six months, and Dudley et al. (1980) reported the organism from sludged fields. Akin et al. (1978) states that Mycobacterium is highly resistant to environmental stresses, and Beard (1938, 1940) believes that this resistance is due to the high lipid content of the cell. Greenberg and Kupka (1957) confirmed the above by noting that Mycobacterium can remain alive in soil from 150 days to 15 months, while Carson et al. (1978) and Ramirez and Malina (1980) were able to isolate Mycobacterium at high levels from sludge, finding that the organisms were resistant to chlorination and liming. Huekelekian and Albanese (1956) and Jensen (1954) record that those individuals with tuberculosis pass the causative agent in the faeces and thence to sewage and sludge and that virulent forms could be found from 11.5 to 15 months afterward.

3.2.4 Growth on Soil and Mechanisms of Destruction

Miller (1974) among others (Hinsley et al. 1972; Edmonds 1976; Temple et al. 1980) recorded that, after placement of sludge on land, the numbers of microorganisms increased and ultimately reach maximum populations in one month. After this time, the total numbers decreased. Wray (1975) found that the persistence of salmonellae on land depended upon moderate temperature, the amount of moisture, sunlight, and pH. Gerba et al. (1975) extended this line of work and found that there could be reproduction amongst Salmonella and Shigella in the soil as long as proper organic nutrients were present and both temperature and moisture content were suitable.

McKinney et al. (1958) stated that the survival of Salmonella in sludge was a function of the nutrients available, retention time, and density of the initial population. Akin et al. (1978) noted that bacteria at the surface of soil were destroyed by the effects of sunlight and drying, while those contained in soil were inactivated by drying and extremes of pH distinct from normal. They found that inactivation was proportional to temperature and, if moisture content was adequate while the temperature was low, the time for survival was extended. Elliott and Ellis (1977) concurred, noting that bacteria in sludged or effluent treated soils are adversely influenced by sunlight, pH extremes, temperature, and drying. As previously mentioned, Bell (1976) and Oldham (1973) found that longevity is influenced by sunlight and moisture. Geldreicht et al. (1978) found that in water (and conceivably sludge) bacterial regrowth can occur with a temperature above 13°C and a pH less than 10 and if available nutrients are adequate, particularly nitrates and organic carbon. McCambridge and McMeekin (1981) noted that visible light caused a decline in numbers of faecal bacteria but not upon their predators and that the combined action of light and predation was significantly greater than that afforded by the sum of the two individual events. Chamberlin and Mitchell (1978) had previously suggested that visible light only injured the bacteria and rendered them more susceptable to predation.

In addition to physical deterrents to microbial growth in soils, certain biological phenomena have been found to be of importance. Gerba et al. (1975) cited the significance of antagonistic soil forms. Pratt et al. (1978) found that most pathogens do not compete well with other soil organisms and are subject to attack by antagonistic soil species. Walker et al. (1977) stated that the survival of Salmonella spp. is inversely proportional to the concentration of indigenous organisms. Guthrie et al. (1976) were more specific in defining at least one competitor when they found that Salmonella and Escherichia coli had a faster rate of decline in the presence of Flavobacterium spp. Experimentally, Hutchinson et al. (1943) showed that certain antagonistic organisms (Pseudomonas, Actinomyces, Sarcina, and Micrococcus) when present in abundance can reduce the numbers of Escherischia coli from 28 to 97% depending upon the combination of mixed strains. Not only can normal soil forms contribute an antagonistic effect but there can exist a synergistic antagonism as well.

Roper and Marshall (1977, 1978) and McCambridge and McKeekin (1981) found that the myxobacter, Polyangium, was capable of lysing various enteric bacteria in sea water, and Roper and Marshall (1977) also observed ciliate and amoeboid protozoans acting as predators of enteric organisms.

The decline of enteric forms in the presence of soil species may be accounted for by one or more of the following reasons. The first is the ingestion and subsequent digestion by protozoans or multicellular animals. In partial opposition, Chang et al. (1960) found that, in water, enteric bacteria ingested by free-living nematodes were protected against high chlorine concentrations even when 90% of the worms were dead. It is conceivable that similar protection could be afforded to soil bacteria against the vagaries of physical environmental changes. Tracy et al. (1966) recorded similar data with crustaceans acting as protective organisms. The various mites (also arthropods) found in soil might be able to act in a similar capacity.

The second reason is the failure of enteric forms to compete successfully with other organisms for available nutrients. This was demonstrated experimentally by Reitter and Seligmann (1947) who found that, when placed in lactose broth, many non-coliforms can multiply more rapidly and simply outgrow the coliform population, thereby causing inhibition.

A third factor is longer generation times resulting in overgrowth by competitors and the possibility that the metabolic end products of overgrowth could be either lethal or produce static populations of the enteric forms.

Additionally, destruction of enteric forms by antibiotics yielded by soil forms may occur, and, possibly, bacteriocins could result in the demise of enteric forms. Bacteriocins are produced by members of the Enterobacteriaceae (among others) and generally have an adverse effect on closely related taxonomic and metabolic groups, resulting in a reduction in competition. It is conceivable that, in soil, bacteriocins may possess a broader range of action than has been previously indicated.

As noted above, there are a variety of factors which are capable of influencing the numbers and detection of coliform bacteria in water, wastewater, and presumably soil. Additionally, sublethal injury (Geldreich et al. 1978; Ray and Speck 1973) may occur in excess of 90% of indicator bacteria when exposed to native water for less than one week (Bissonnette et al. 1975, 1977) and organisms debilitated in this fashion often are unable to colonize certain selective media (Maxey 1970).

Aside from a variety of physical and biological features causing stressed bacteria, the employment of selective media may be responsible for decreased efficiency of indicator bacteria recovery (Bissonnette et al. 1977; Dutka et al. 1979; Dutka and Tobin 1976; Grabow and du Preeze 1979; Hoadley and Cheng 1974; Ray and Speck 1973; and Schusner et al. 1971). Bacteria damaged by exposure to water are even more sensitive when added to selective media. Bissonnette et al. (1977), Dutka et al. (1979), and Zaske et al. (1980) are of the opinion that such poor growth is a function of cell wall damage.

McFeters et al. (1982) compared the influence of a variety of selective media upon the recovery of normal and injured coliforms (Escherichia, Klebsiella, Enterobacter, and Citrobacter). In the case of lauryl typtose broth, an average of 56% (34 to 79%) of damaged cells were recovered, whereas MacConkey agar yielded but 5% of the stressed organisms applied; m-FC broth at 44.5 °C showed suitable growth in but 7% (4 to 10%) of the instances.

3.2.5 Adsorption of Bacteria by Soil

The question of the mobility of bacteria when applied to soil with sludge is important because of the possibility of percolation to groundwater. It also pertains to the procedures for the analysis for various species from sludged soil samples (i.e., is it necessary to analyse samples from various depths, or is there a depth below which few if any organisms will have migrated?). Krone (1968) states that bacterial removal in soil is mainly the result of soil filtration/straining at the soil surface, and Wilson and

Beckett (1968) and Christman (1972) believe that most microbes are removed in the top few centimetres of soil. Dazzo et al. (1972) found that most faecal coliforms were immobilized in the top 3 to 4 cm of soil, and Brouwer et al. (1974) noted the same for total coliforms. Gerba et al. (1975) and Reneau and Petty (1975) believed that most bacteria were adsorbed in the top 3 to 4 cm of soil. Edmonds (1976) found that few bacteria contained in sludge applied to soil penetrated more than 5 cm to the underlying soil and felt that groundwater contamination from vertical movement was unlikely though a few faecal coliforms "were recorded in the groundwater, generally being less than 5/100 mL and mostly 0/100 mL." Edds and Davidson (1981) found that 90% of *Escherichia coli* were removed from the fluid phase when mixed with a 10% soil suspension, and that a sludge concentration of 4% did not affect the ability of soil to adsorb bacteria. Finally, Scheraga et al. (1979) believed that the attachment of bacteria to soil was caused by physical forces, and that non-motile bacteria (*Flavobacterium* and *E. coli*) sorbed equally as well to a clay-sludge mixture as to sand. Motile bacteria (*Aeromonas*) adsorbed to the clay-sludge mixture twice as readily as to sand.

3.2.6 The Secondary Pathogens

A number of organisms producing classically important diseases (primary pathogens, e.g., tuberculosis, salmonellosis) in man are found in sewage waste disposal products. In addition, certain bacteria that have long been considered to be benign have recently been shown to be both pathogenic (Center for Disease Control 1978) and capable of existing in sludge. These bacteria may be referred to as secondary pathogens (those which attack immunologically weak hosts). Guentzel (1980) reports that the following genera of the Family Enterobacteriaceae fall into this category--*Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter*--and reported them from effluent. These opportunistic pathogens are responsible for health problems throughout the world.

A study by the Center for Disease Control (1978) preconfirmed the above genera as being of significance (except for *Citrobacter*) and extended the list by the addition of *Proteus*, *Providencia*, *Pseudomonas aeruginosa*, *Staphylococcus*, and group D *Streptococcus* (faecal streptococci).

The replacement of classically significant pathogens with other species previously considered of secondary importance is neither a new nor an unusual concept. For instance, Sekla (1970) in Manitoba noted that, as the incidence of typhoid and paratyphoid fevers decreased, there was a rise in the numbers of cases of other forms of salmonellosis. Healy and Visvesvara (1977) similarly report that, as numbers of water borne cases of amoebiasis decreased, the void was filled with increased numbers of cases of giardiasis.

The organisms under question, then, are Enterobacteriaceae and fall within the following genera: *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus*, and *Providencia*. The genera *Edwardsiella* and *Hafnia* have not been implicated as significant pathogens. In addition to producing human infections, these secondary pathogens can, because of their endotoxins, act as allergens capable of eliciting pathological changes upon inhalation. Regarding the significance of bacterial endotoxins, Mattsby and Rylander (1978) and Rylander et al. (1976, 1979) reported both clinical symptoms and immunological modifications amongst workers dealing with heated sewage sludge. Concern was expressed that such reactions were due to bacterial endotoxins inhaled with the dust derived from such sludge. Subsequently, Lundholm and Rylander (1980) described similar reactions from workers in a plant where municipal sludge and garbage were crushed and milled before being composted. Large numbers of Gram negative bacteria (those which produce endotoxins) were found to be present. In a laboratory study, Rylander (1978) found that inhalation of endotoxin (lipopolysaccharide) caused an acute inflammatory response in the lungs of mice and speculated that long-term exposure could lead to pathological manifestations of lung tissue. Clark et al. (1981) stated that the health hazard of exposure to microbial pathogens and their toxins present at sludge composting (or spreading) sites has not been well investigated. They then investigated the immunological responses of workers at sludge composting areas and found abnormal reactions which were attributed to the inhalation of bacterial endotoxins.

Evans (1975) found that enteric bacteria (as opposed to total coliforms) yielded the greatest quantity of endotoxins, followed by total coliforms and other Gram negative bacteria. The other heterotrophic bacteria present contributed two orders of magnitude less endotoxin. Inasmuch as the above-noted members of the Enterobacteriaceae may act not only as invaders but as allergens, it is important to know the numbers present in sludge and sludged soil as well as the relative proportions of genera so that contaminated soil (sludged) may be compared with unsludged soil.

Dudley et al. (1980) and Harding et al. (1981) reported secondary pathogens from sludge and aerosols produced during sludging of land and also found both *Hafnia* and *Yersinia* as well (both belong to the Enterobacteriaceae). From the data presented, the percentage attributed to each species or organism were determined. Examination of these data indicates that there is a wide disparity amongst the figures reported for each genus at separate samplings and that the primary pathogens (*Salmonella*, *Shigella*, and *Yersinia*) are designated as representing "none," "few" or a small percentage of the total. Even though there is a disparity in percentages between samples, a perusal of the average values reported in Table 4 shows that the following genera rank fall into the top four: *Klebsiella*, *Escherichia*, *Citrobacter*, and *Enterobacter*. Burge and Millner (1980) reported the genera *Serratia* and *Pseudomonas* (not a member of the Enterobacteriaceae) as secondary pathogens from composted sludge. Sagik et al. (1980) state that *Enterobacter*, *Klebsiella*, *Proteus* and *Providencia* (in addition to *Mycobacterium* and *Staphylococcus*) are received in significant numbers by sewage treatment plants and that the rate of their transmittal to land application sites is a function of the efficiency of the treatment operation. Guentzel (1977) considers *Klebsiella* to be an especially important pathogen but there is no simple or reliable method for quantitation. Eller and Edwards (1968) have described a nitrogen deficient medium for the isolation of both *Klebsiella* and *Enterobacter* but the procedure could not be successfully implemented by the Kananaskis Laboratory.

Although not directly applicable to this study, information is available as to the percentages of the genera of the Enterobacteriaceae generated from effluent spray irrigation aerosols (Johnson et al. 1977; Clark et al. 1981) as well as from effluent (Guentzel 1977). These data are presented in Table 5. The types, though perhaps not the numbers, of coliforms present in

Table 4. Average percentages of Enterobacteriaceae from digested sludge[a].

Organism	n	Mean	S.D.[b]
Escherichia	9	16.2	14.3
Shigella	9	few or none detected	
Edwardsiella	9	0	0
Salmonella	9	few or none detected	
Arizona	9	0	0
Citrobacter	9	12.2	15.6
Klebsiella	9	35.9	21.9
Enterobacter	9	19.5	23.0
Hafnia	9	8.6	16.3
Serratia	9	4.6	5.0
Proteus	9	0.3	0.4
Providencia	9	0	0
Yersinia	9	1.7	2.4

[a] Compiled from Harding et al. (1981) and Dudley et al. (1980).

[b] Standard Deviation.

Table 5. Percentages of pathogens present in effluent spray irrigation aerosols.

Organism	Aerosol[a]	Aerosol[b]	Effluent[c]	Mean % Enterobacteriaceae from Aerosols
Escherichia	3.0	5.0	7.2	5.1
Shigella	0.1	0	2.9	1.0
Edwardsiella	0.1	0	0	0.03
Salmonella	0.1	1.7	0.7	0.8
Arizona	0.1	0	0	0.03
Citrobacter	3.0	10.7	16.6	10.1
Klebsiella	3.3	25.6	10.1	13.0
Enterobacter	90.1	52.9	28.2	57.1
Hafnia	0	0	0	0
Serratia	0.1	4.1	2.8	2.3
Proteus	0.1	0	24.6	8.2
Providencia	0.1	0	0.7	0.3
Unidentified	0	0	5.8	1.9

[a] From Johnson et al. (1977).

[b] From Clark et al. (1981).

[c] From Guenzel (1977).

effluent are probably similar to those found in raw sludge. The percentages of each genus would be modified by the effects of anaerobic, mesophilic digestion.

Regarding total numbers of total and faecal coliforms/g in sludge, Dudley et al. (1980) found an average of 2.3 x 10^7 total coliforms, and 2.8 x 10^6 faecal coliforms. Harding et al. (1981) noted a range of 2.1 x 10^5 to 6.1 x 10^7 for total coliforms in 12 samples. For faecal coliforms, the figures were 1.2 x 10^4 to 7.8 x 10^6. Edmonds and Littke (1978) reported 3.5 x 10^7 total coliforms and Edmonds (1976) later found total coliform counts of 1.4 x 10^6 to 1.9 x 10^6/g of sludge. Other workers have reported on the numbers of total and faecal coliforms present from municipal solid waste disposal sites (Gaby 1975; Peterson and Stutzenberger 1969; Peterson 1971; and Cooper 1974) and although such data are not particularly useful to this review, the numbers reported are high and do indicate that coliforms as a group should be of concern at landfill sites.

3.2.7 Numbers of Other Pathogens in Sludge

3.2.7.1 *Mycobacterium*. Regarding the presence of *Mycobacterium* spp. in sludge, Harding et al. (1981) made 12 determinations, two from each of six sludge sites, and found from less than 1.1 x 10^6 to 2.4 x 10^9 acid fast organisms/g. When dealing with raw sludge the number was 1 x 10^9 which fell to 1 x 10^8 in anaerobically digested sludge. Dudley et al. (1980) recorded an average of 8.3 x 10^8 cells/g in sludge of *Mycobacterium* spp.

3.2.7.2 *Staphylococcus*. Harding et al. (1981) found *Staphylococcus* in but 4 of 12 sludge analyses with an average number of 5.6 x 10^6/g, while Dudley et al. (1980) noted the organisms in but one of three examinations at levels of 1.2 x 10^6/g.

3.2.7.3 *Pseudomonas*. With respect to fluorescent *Pseudomonas*, Harding et al. (1981) reported an average of 1.4 x 10^4 (ranging from 6.6 x 10^2 to 3.7 x 10^5)/g sludge, while Dudley et al. (1980) found 6.4, 2, and 7.1 x 10^4/g.

3.2.8 Numbers of Bacteria Required to Infect

The numbers of microorganisms required to produce a patent infection in humans are not well known and are affected by the general and immunological health of the individual involved. Sorber et al. (1972) state that little is known of the minimum infective dose of most pathogens and that the range could be from one to thousands. Sagik et al. (1980) mention that few data are available regarding minimal infective doses except for those dealing with healthy human volunteers challenged with laboratory strains. Hoadley and Goyal (1976) report that 10 to 100 *Shigella* can yield an infection while the numbers required to cause salmonellosis vary between 10^4 and 10^9. McCullough and Eisele (1951a, 1951b, and 1951c) determined the doses required to infect 50% of volunteers using five species of *Salmonella*. They found that infective dose data are not available for *Salmonella typhimurium*, the organism most commonly reported in the United States. Taylor and Burrows (1971) reported that the infective dosage for *Salmonella* exceeds 10^6 organisms.

4. METHODS

4.1 SAMPLING LOCATIONS

Sample collections for parasitological and bacteriological analysis were made from the following locations:

1. The activated sludge plant at Bonnybrook, Calgary. Samples were taken from raw sewage, the anaerobic digester (38° to 40°C), and liquid effluent.
2. The lagoon facility in Crossfield. Samples were taken from:
    - (a) the anaerobic digesting pond which was receiving the raw sewage;
    - (b) the two month aerobic lagoon;
    - (c) the final 12 month-aerobic holding lagoon; and
    - (d) at Crossfield, samples were also taken from a dairy cattle yard to provide base line data for pasture land continually occupied by small numbers of animals. To supplement the above, soil samples were taken from a larger pasture, and from grassland which had been spray irrigated from the 12-month holding lagoon during the fall before sampling.
3. Sludge lagoons and agricultural land near the Shepard sludge lagoons which had been treated with sludge from those lagoons, including fields sludged in 1978, 1979, 1980, 1981, and 1982 (the latter applied to grass).
4. Farmland owned by Mr. Bob Copithorne some 10 miles west of Calgary including:
    - (a) a moderately to heavily grazed cattle pasture; and
    - (b) a hayfield, ungrazed for a least three years, which served as a control for sludged fields and thus provided normal background data.
5. Buried sludge including the following:
    - (a) a 20 L sample of lagoon sludge applied at a depth of 20 cm and then covered. This sample was located in an unsludged area of the 1981 field and done in 1981.

(b) sludge which was machine injected in 1982 at a depth of 15 cm by the City of Calgary.

6. Raw sludge and 38°C digester sludge from the Fish Creek plant.

4.2 METHODS OF SAMPLING

All samples for analysis were placed in sterilized glass jars and returned to the laboratory in an ice chest. Soil and sludge samples were scraped from the surface or lower depths with sterilized spoons. Liquid samples were dipped from the surface, and bottom deposits from the lagoons were taken with a long-handled "pooper-scooper." Digester samples were taken from a main tank spigot.

4.3 METHODS: PARASITES

4.3.1 Parasite Analysis

Not all parasite contaminated material responds identically to the same method of analysis. For example, nematode eggs and protozoan cysts can be collected and concentrated by flotation methods but the eggs of tapeworms and operculated trematodes must usually be searched for by sedimentation (McDonald 1920; Faust and Russell 1964), because of differences in specific gravity and fragility. The methods outlined below describe the techniques used to find parasites in raw sewage and sludge, manure, digestion sludge, effluent, lagoon sludge, sludged soil samples, and buried sludge.

Several methods of analysis for parasite stages were tested before the final procedure ($NaNO_3$ flotation, sp. g. 1.25) was accepted as the definitive way to proceed. The reason for experimentation with a variety of procedures was that clinical methods abound and each has both advantages and disadvantages. Information dealing with the extraction of parasites stages from sludge, soil, or sludged soil, however, is not as readily available since this type of analysis constitutes a relatively new phase of investigation (i.e., environmental parasitology). Early methods then were tried and discarded in favour of new methods until the best possible was achieved for this new type of work.

4.3.1.1 Raw Sewage and Effluent. Samples of raw sewage were obtained from the anaerobic digestion lagoon in Crossfield and samples of raw sewage and effluent from Bonnybrook, Calgary. One litre of sample was evenly distributed in 250 mL centrifuge tubes and centrifuged at 2000 rpm for two minutes at room temperature in a Sorval RC-5 refrigerated centrifuge. If still turbid, the supernatant was poured off and replaced with tap water and the process repeated until it was clear. Finally, most of the liquid was removed and the sediment was mixed with residual liquid using a pipette. All samples were then combined, placed in a 15 mL centrifuge tube and diluted to 10.5 mL. A 0.1 mL sample of well mixed suspension was then transferred to a haemocytometer; 5 of these 0.1 mL samples were counted and final numbers and types of parasites per L were determined by applying appropriate dilution factors.

The remaining sediment sample (10.0 mL) was used to provide material for the $ZnSO_4$ flotation concentration method described by Faust et al. (1938, 1939). Another method, sucrose flotation (Palmieri and Andersen 1979), was also tested. The liquid containing the parasites was centrifuged at 2000 rpm for one minute in an International Clinical Centrifuge and the supernatant poured off. Four to 5 mL of sucrose solution (sp. g. 1.16) was added and the sediment broken up and mixed evenly with a stirring rod. Then, more sucrose was added, with stirring, until the 15 mL mark was reached in the centrifuge tube. This was followed by centrifuging at 2000 rpm for one minute and then sucrose was added until the liquid formed a convex surface upon which a cover glass was placed and allowed to remain for 10 minutes. Cysts and eggs which rose to the surface adhered to the cover slip. The cover slip was then removed, placed on a glass slide, and the numbers and types of parasites were counted. Successive flotations were carried out on the same sample until no parasites were found in two successive observations; 3 to 4 counts were generally sufficient to achieve this end.

4.3.1.2 Raw and Anaerobically Digested Sludge. Because of the high solid content of this material the methods described above did not yield very many parasites, presumably because the cysts and eggs adsorbed to the solid fraction and were not readily floated.

A method of freeing the parasite material using antiformin (1.8% NaOCl) was attempted because of the success achieved with this method by Spindler (1929) and Graham (1981). The results were more satisfactory but another method was devised which exceeded the results yielded by the antiformin method. This technique is described below.

Two mL of well-shaken sludge were placed in a 15 mL centrifuge tube along with 0.5 g of commercial detergent soap ("Cheer"). The sample was thoroughly mixed with tap water and then allowed to stand for 20 minutes. Centrifuging at 2000 rpm for 1 minute and replacement of cloudy supernatant with tap water followed until the supernatant was clear. The final concentrated sample was floated with sucrose as mentioned above and the total number and types of parasites per 2 mL sample determined. Because of the large amount of residue remaining, this method was not acceptable for use as a sedimentation method for tapeworm eggs, although 1:10 dilutions of the sediment yielded some eggs. In addition, whole, well-mixed sludge was diluted 1:10 with tap water and 0.1 mL of the sample was placed on a slide and covered with a 22 x 40 mm cover slip and the types of parasites noted by direct observation. This method was eventually supplanted by the $NaNO_3$ flotation method described under the section on soil-sludge mixtures.

4.3.1.3 Lagoon Sludge. Sludge from the lagoons, which had a higher solid content than that from the anaerobic digester, was treated in a fashion similar to that described for the plant digested material, except that only 1 g was used as a sample. Flotation methods were quite acceptable but, again, sedimentation was hampered by the concentration and density of the sediment. Dilutions (1:20) with water of either sediment or whole sludge gave better results. Further improvements were obtained using the $NaNO_3$ flotation method described below.

4.3.1.4 Soil-Sludge Mixtures. Initially, 1 g samples of soil-sludge mixtures were processed as described for the sludge from lagoons, and flotation methods gave good results. Because of the amount of particulate material present, neither sedimentation nor direct analysis methods gave good results regardless of the methods employed (i.e., water mixture, antiformin, or commercial detergent soap).

Prior to initiating random sampling/coring studies, the effectiveness of flotation methods was investigated. This was prompted by a publication (O'Grady and Slocombe 1980) which critically analysed the value of $NaNO_3$ as a flotation agent for the eggs of veterinary parasites. Because of the careful work and excellent results achieved by these authors, it was decided to compare sucrose flotation (which had been used in our earlier work) with $NaNO_3$ flotation.

Horse strongyle eggs obtained locally were concentrated and the liquid (0.2 to 0.4 mL) containing a known number of eggs was added to 5.0 g of sterile 1978 field soil and mixed with a spatula. Half a mL of tap water was then added and, after mixing, the stiff mud was allowed to stand for 20 minutes. The number of eggs per g of soil ranged from 300 to 700 for different repetitions. One-tenth g of detergent ("Cheer") and 10 mL of tap water were added to the sample which was then stirred for 5 minutes. The mixture, together with subsequent washings of the container, was then equally distributed into two 15 mL centrifuge tubes. After centrifuging at 2000 rpm for one minute, the supernatant was poured off and mixed with either sucrose (sp. g. 1.16) or $NaNO_3$ (sp. g. 1.25). These were centrifuged as before; appropriate flotation fluid was added until a cover glass could be floated on the meniscus and, after 15 minutes, it was removed for examination. More flotation fluid was added and a second cover slip was put in position and allowed to remain for 20 minutes when it, too, was examined. Eight repititions were used for sucrose and 11 were carried out for $NaNO_3$. The results of this comparative work showed that $NaNO_3$ recovered approximately 8 $\pm$0.9% of the added eggs as opposed to sucrose which only recovered 4.5 $\pm$0.7%. In addition, less debris was floated up with $NaNO_3$, giving a clearer counting field. No significant differences were observed between the recovery of fresh and formalized eggs. Although this recovery is low, it was consistent and compares well with other published results (O'Grady and Slocombe 1980). Flotation with $NaNO_3$ then was selected as the method of choice for all later work.

In the subsequent random sampling/coring analyses, two 2.5 g samples were taken from a larger sample and treated as described above. The results of the paired samples were averaged when determining the egg concentration per g, and samples for each section of each site were dried so that number of eggs per kg of dry weight sample could be reported. Analysis of 2.5 g samples with two consecutive flotations per single tube was subsequently adopted for all future parasite work.

4.3.1.5 Analysis for Infective Larvae of Parasitic Nematodes. The third-stage, infective larvae of parasitic nematodes could be recovered during the flotation analyses of sludge, sludged soil, and animal manure. Often, though, they appeared shrunken and distorted presumably because of the osmotic nature of the flotation medium. Additionally, the larvae noted represented only those which were in that stage at the time of flotation and the forms still contained in and developing within eggs were discounted.

In the third year of this study, it was considered necessary to make a more concerted effort to find the types and numbers of larvae contained within the sludge/soil matrix in order to make comparisons between the parasites found at the slaughterhouse and those found on sludged fields. This was done by the use of the Baerman apparatus, together with sequential observation of an incubated matrix.

The Baerman apparatus (Figure 1) is a simple piece of equipment, the use of which involves placing material containing parasites on cotton gauze suspended in a fine screen wire concavity on a funnel. After covering the matrix to approximately one half its height with warm (40°C) water, a period of about 12 hours (or overnight) is allowed to pass. The liquid at the bottom of the clamped rubber tube at the bottom of the funnel is drained into a centrifuge tube and the worms centrifuge concentrated (2000 rpm for 30 sec.). All of the liquid, except about 1.0 mL, is poured off; the residue is mixed by pipette aspiration and an aliquot placed on a slide, covered, and examined. In order to stop active motion so that details may be observed, the preparation may be briefly exposed to a Bunsen burner flame in order to kill the organisms and leave them in an extended position so that the internal morphology may be more readily observed.

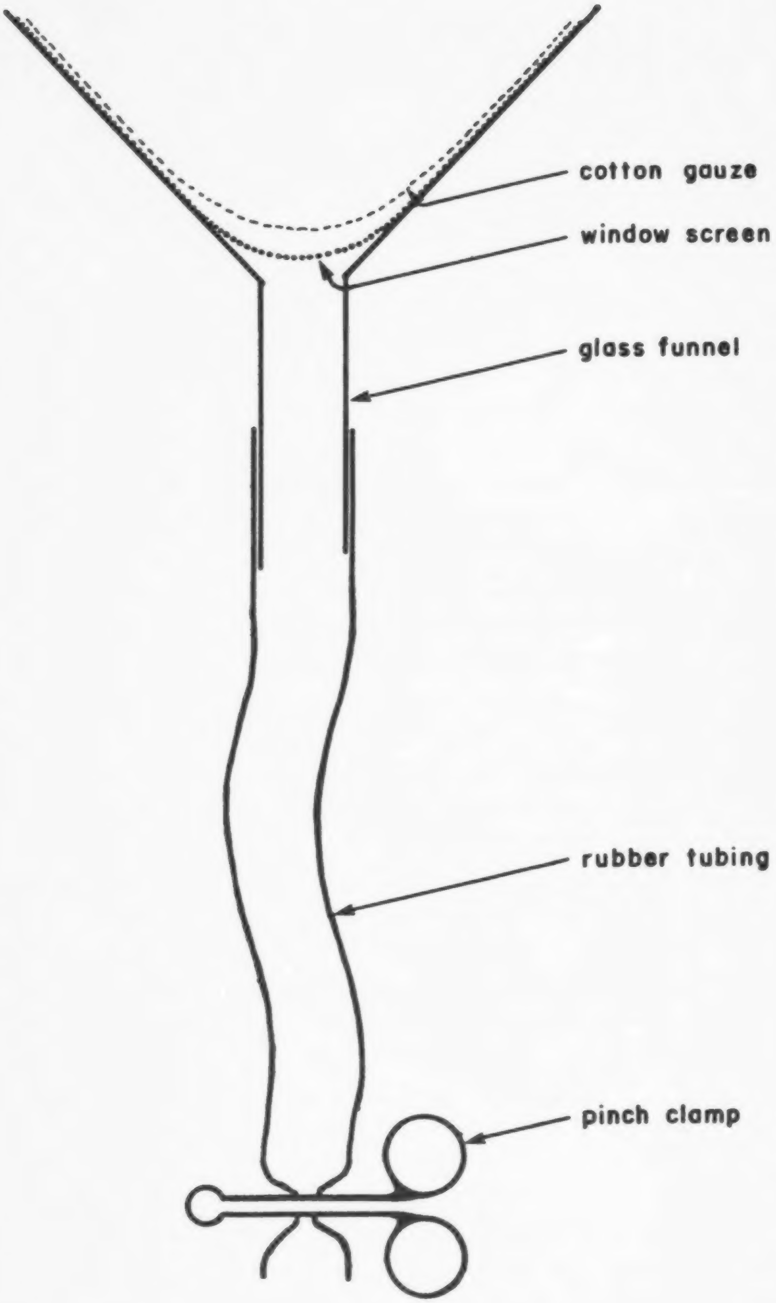


Figure 1. Baerman apparatus.

One theory behind the success of the Baerman apparatus is that the warm water will simulate the body temperature of the host, causing the parasites to become active, wiggle free in the liquid, and sink to the bottom. Alternatively, regardless of temperature, nematodes are quite active in a liquid soil situation and some will reach the water where they will sink to the bottom simply because their musculature is inadequate to keep them suspended in the water column.

In this work, samples were analysed by flotation and those with the largest number of eggs or worms (free-living adults, larvae, or parasitic stages) were removed in 20 to 25 g amounts and mixed. Ten g quantities of the mixtures were placed in the Baerman apparatus and examined the following day; all of the remainder of the matrix was placed in a dish, moistened if necessary, covered, and allowed to incubate at room temperature for seven days after which portions were again placed in the Baerman apparatus.

4.4 METHODS: BACTERIOLOGY

4.4.1 Indicator Organisms and Enteric Pathogens

Samples used for bacteriological studies were the same as those obtained for parasitological analysis. For indicator organisms, the following techniques were employed.

4.4.1.1 Total Coliforms, Most Probable Number (MPN) Method. Dilution blanks of 10^{-4} to 10^{-6} were made and a series transferred to lauryl tryptose broth. If gas was shown at 35 to 37° C in 24 to 48 hours, a positive presumptive test was assumed. Subsequently, portions of the positive tubes were transferred to brilliant green lactose broth where gas formation indicated a confirmed test for total coliforms. This method was eventually abandoned in favour of MPN faecal coliforms.

4.4.1.2 Faecal Coliforms (MPN). Dilutions of sample were placed in lauryl tryptose broth at 37°C and aliquots of those tubes displaying gas were transferred to EC broth which was incubated at 44.5° ±0.2°C. Gas formation is indicative of a confirmed test for faecal coliforms and the MPN is determined from tables depending upon the number and dilution of the positive tubes. Initially 10^{-4} to 10^{-6} dilutions were used but in studies on sludged fields, where faecal coliforms were only occasionally found, 10^{-1} to 10^{-3} dilutions were employed.

4.4.1.3 Faecal Streptococci (MPN). Dilution blanks of 10^{-4} to 10^{-6} were prepared and portions inoculated into azide dextrose broth incubated at 35 to 37°C for 24 to 48 hours. A positive presumptive test is indicated by growth in this medium. Growth of several loops of transfer material in ethyl violet azide glucose broth constitutes a positive confirmed test as determined by the MPN method.

4.4.1.4 Faecal Streptococci (Pour Plate Method). Samples of the above noted dilutions were placed in a Petri dish and then mixed with KF agar to which 1% TTC was added immediately before pouring. After 48 hours at 35 to 37°C, the total number of suitably coloured colonies was counted and total numbers determined according to dilution. Both faecal streptococcus methods were abandoned and replaced with MPN faecal coliform analyses.

4.4.1.5 *Clostridium perfringens*. *Clostridium perfringens* was cultured on SPS agar. One tenth mL samples were spread with an L-rod on the surface of a poured plate and, after allowing 30 minutes for absorption, 20 mL of SPS was overlayed to promote anaerobic conditions. The culture was incubated at 35 to 37°C for 48 hours. After 48 hours the black colonies were counted and the appropriate dilution factors used to determine numbers/unit volume. Results were contradictory and confusing; this procedure was superseded by the MPN faecal coliform procedure.

4.4.1.6 Enteric Pathogens. The method of detection employed followed standard laboratory techniques with certain modifications interspersed to negate those species superficially resembling *Salmonella* in the initial stages of diagnosis. Selective enrichment in selenite broth was followed by streaking on selective agar media (bismuth sulphite). Characteristic colonies (a maximum of 10) were transferred to triple sugar iron agar and those with the proper growth patterns were then streaked onto phenylalanine agar slopes (to rule out Proteeae), inoculated into lactose broth to eliminate slow lactose fermenters, and anaerobic glucose broth to select out *Pseudomonas* and, additionally, oxidase and nitrate reactions were determined. The organisms selected by these means may be *Salmonella* spp. and can be further identified by a series of biochemical and serological tests.

4.4.2 Primary and Secondary Bacterial Pathogen Analyses

During the third and final year of the project, greater emphasis was placed upon the isolation and determination of primary and secondary bacterial pathogens and allergens. The following methods were used.

4.4.2.1 *Sampling*. For the fields, 15 samples of approximately 100 g each were collected by a random sampling method from the top 2.5 cm of ground. Ten g of each sample was stirred with the others and mixed in a blender for 30 seconds after which 15 g was removed and placed into 150 mL of sterile water; this 15 g/150 mL subsample served as a source for all bacteriological examinations. For sludge, 15 mL was placed in a 150 mL water blank. The remainder of the soil and sludge samples was kept and examined for parasites and served as a source for Baerman analyses.

Regarding the buried sludge, a single sample of approximately 100 g was taken, of which 15 g was added to 150 mL of water and treated as above. The remainder served for parasitological examinations.

4.4.2.2 *Total Plate Count*. 0.1 mL of 10^{-4} to 10^{-8} dilutions of the mixed subsample were added to the surface of each of three poured, hardened nutrient agar plates (i.e., three replicate plates for each dilution). Each aliquot was spread with an L-rod and allowed to incubate at 37°C for 30 to 36 hours. Colonies were counted on those dilution series showing from 10 to 100 colonies to determine the total background plate count of those organisms capable of growth on nutrient agar at 37°C.

4.4.2.3 *MacConkey Plate Count*. This analysis is done to determine the numbers of Enterobacteriaceae from each combined sample capable of growing upon the surface of a moderately selective agar. This method allows for the growth of Gram negative bacterial colonies while excluding colonization by Gram positive organisms. The organisms included can be oxidase positive or negative (Enterobacteriaceae), and the proportions of the two groups can be determined by the application of the oxidase test.

The oxidase negative reaction is not restricted to the Enterobacteriaceae and certain organisms capable of growth on MacConkey agar are likewise oxidase negative. *Erwinia*, although it belongs to the Enterobacteriaceae, is generally associated with plants as a pathogen or saprophyte (one species is designated as a human parasite). Members of this genus must be distinguished from the other Enterobacteriaceae, particularly since they can be encountered from agricultural fields. This can be accomplished by determining whether or not bacteria are capable of reducing nitrate to nitrite. All of the Enterobacteriaceae are capable of this reaction except *Erwinia*; *Pectobacterium* is nitrate positive but it generally fails to produce acid *and* gas from glucose. Ewing and Martin (1974) state that with *conventional* tests *Erwinia* fail to produce this reaction, though Buchanan and Gibbons (1974) simply state that some of the *Erwinia* are capable of reducing nitrate to nitrite. Ewing, however, is a recognized authority on the differentiation of the Enterobacteriaceae and his expertise must be recognized.

There are, in addition, certain other Gram negative, oxidase negative organisms capable of growth on MacConkey agar which conceivably could be encountered and could confuse the results of the Enterobacteriaceae count to a limited extent. Their major interference, however, would be felt on the determination of genera considered in this study, and methods were applied for their detection. Tatum et al. (1974) have reviewed these organisms and it appears that there are four groups of bacteria which must be considered. Some species of *Acinetobacter* capable of growth on MacConkey agar fail to use carbohydrates (glucose) either oxidatively or fermentatively and, because they are strict aerobes, they can be detected from their failure to grow at any distance below the surface of routine, diagnostic stabs. In addition, they are nitrate negative. Other species of the same genus can use glucose oxidatively but will fail to grow to any depth in stabs and are also nitrate negative. Some other glucose oxidizers, designated Ve biotypes 1 and 2, are considered to be *Pseudomonas*-like and possibly *Xanthomonas*. Even though some 63% studied are capable of reducing nitrate, they are also strict aerobes and will fail to display any subsurface growth in stabs.

A group designated HB-5 are possibly a form of *Flavobacterium* capable of growth on MacConkey agar, although they require from 5 to 7 days for colonies to appear.(Our assays using MacConkey agar were not incubated beyond 48 hours.) They, like the Enterobacteriaceae, are fermentative and are capable of reducing nitrate; about 41% can be construed as being oxidase negative, though Tatum et al. (1974) state that they are actually weakly oxidase positive.

The key which follows indicates those characters which were employed to differentiate the MacConkey positive, oxidase negative forms which could confuse the study of the Enterobacteriaceae unless distinguished:

I. Oxidative ----------------------------------*Acinetobacter*
Ve biotypes 1 and 2

II. Fermentative
  1. Do not reduce nitrate to nitrite------*Erwinia*
  2. Reduce nitrate; require 5-7 days for growth on MacConkey agar------------------------Group HB-5

The method employed was similar to the total plate count. Serial dilutions were made on the surface of three MacConkey agar plates employing dilutions of 10^{-2} to 10^{-6}. After incubation at 37°C for 36 to 48 hours, dilution plates showing 10 to 100 colonies were counted and Gram stains of representative colonies were made to affirm that Gram negative forms were present. To determine the oxidase reaction, the plates were flooded with N, N-dimethyl-p-phenylene diamine solution and, after five minutes, the numbers of oxidase positive (blue-black) and oxidase negative (no colour change) colonies were recorded.

4.4.2.4 Identification of the Enterobacteriaceae. 1.0 mL of the original subsample was added to each of seven lauryl tryptose broth tubes and incubated for 24 hours at 37°C. From each of these tubes, a MacConkey agar plate was streaked and incubated as before. 20

isolated colonies were selected from the streak plates and care was taken to ensure that only the material from the centre of each colony was taken so as to preclude contamination from the surrounding area (which may contain attenuated organisms not capable of colonization on the plate but which could grow later). Each colony was transferred to an LTB tube (after inoculation, each colony residue was checked for the oxidase reaction) and incubated for 24 hours at 37°C. This served as an inoculum for the following media which were used to identify the organisms:

1. SIM (tests for motility, indol production and H_2S production);
2. MR-VP medium;
3. Simmon's Citrate slopes;
4. Phenylalanine slopes;
5. Urea broth;
6. Nitrate slope;
7. Ornithine broth;
8. Lysine broth;
9. Nutrient gelatin;
10. Malonate broth;
11. Purple glucose broth;
12. Purple lactose broth;
13. Purple sucrose broth;
14. Sorbitol broth;
15. Triple sugar iron agar slopes (for characteristic reactions, and as a source of material for a terminal Gram stain and a final oxidase test).

4.4.2.5 Pseudomonas Analysis (MPN). From the original 15/150 dilution, three asparagine broth tubes were inoculated with 1.0 mL each. This was repeated with three additional tubes each from the 10^{-2} and 10^{-3} dilutions. All tubes were incubated at 37°C for 48 hours; after this time, a loopful was transferred from the surface growth of each tube to centrimide agar, incubated for 24 and 48 hours at 37°C, and, at each time interval, read for fluorescence with long length ultraviolet light. After fluorescence had been determined for each of the centrimide samples, the most probable number (MPN) was determined by using the following formula:

$$\text{MPN} = \frac{\text{Number of positive fluorescent plates} \times 100}{\sqrt{(\text{mL sample in negative tubes})(\text{mL sample in all tubes})}}$$

4.4.2.6 Staphylococcus Analysis. From the original sample, 0.1 mL were added to each of five mannitol salt agar plates and smeared over the surface with a glass L-rod. The plates were incubated for 24 hours at 37°C and searched for the presence of yellowish colonies surrounded by a yellow area indicating acid production from mannitol. If colonies were not well defined, they were incubated for an additional 24 hours. If the resulting colonies were Gram positive cocci, they were transferred to brain-heart infusion agar and incubated for 16 to 24 hours at 37°C. To check for coagulase activity, two drops of culture were added to 0.5 mL coagulase plasma (or coagulase plasma EDTA) and incubated at 37°C. Hourly checks were made for coagulation.

4.4.2.7 Mycobacterium Analysis. Fifteen millilitres of the 15/150 dilution were placed into each of two sterile centrifuge tubes and centrifuged at 2000 rpm for 20 minutes. The supernatant was removed and replaced with 3% NaOH, stirred and, after 10 minutes, centrifuged for 20 minutes. The supernatant was replaced with a phosphate buffer of pH 7 and centrifuged again. The supernatant was poured off and some of the sediment was inoculated by loop to the surface of Middlebrook slopes (7H9 Agar with OADC). The lids were screwed down tightly and the tubes were incubated at 37°C. If growth occurred, colonies were checked for acid fastness.

4.4.2.8 Faecal Coliforms. The LTB-EC method previously described was used.

5. RESULTS AND DISCUSSION: PARASITES

5.1 CROSSFIELD

5.1.1 The Crossfield Lagoon System

The lagoon system at Crossfield is typical of the newer lagoon treatment facilities being constructed in Alberta. It consists of four small anaerobic lagoons, a two-month holding lagoon, and a 12-month storage lagoon. The system is currently being underused and none of the lagoons was full. Samples were taken of the water and sludge from all three types of lagoon for both parasitological and microbiological analysis. Parasitological examinations of the liquid fraction revealed that *Enterobius* was occasionally isolated from the anaerobic lagoons but, otherwise, the results from the liquid fraction were negative.

The bottom sludge or deposit was sampled from all three types of lagoon using a long handled "pooper-scooper" and subjected to parasitological analysis. Since the 12-month lagoon held the most parasites, it was sampled more intensively than the others. The results of parasitological examination of the bottom sludge of the anaerobic and two-month lagoons appear in Table 5. The sampling sites and numbers of egg/kg found around the 12-month lagoon are indicated in Figure 2. Eggs, particularly strongyle-like, are most abundant in the bottom deposit of the 12-month lagoon, although *Toxocara*, *Nematodirus* and *Enterobius* were also found. Figure 3 shows, by bar graph, the numbers of parasite eggs from all lagoons with particular emphasis being placed on numbers per sampling point for the 12-month lagoon.

Relatively few parasite eggs/cysts (2000/kg) were recorded from the anaerobic pond and, in the bottom deposit of the two-month lagoon, the figure was approximately the same (1600/kg). The situation in the 12-month holding pond departed from the above in that the average number of parasitic forms was higher (6600/kg) and, in addition, there tended to be a distribution pattern of worm eggs which could be accounted for by current flow through the system and prevailing wind patterns. The fact that numbers of eggs in the 12-month lagoon were greater than those noted in the intake anaerobic tank of the 2-month lagoon suggests that eggs are easily transported in water currents but, inasmuch as the 12-month

Table 6. Crossfield parasitology examinations: numbers of parasites per kg of sludge.

	Anaerobic Lagoon: Sludge			Two-Month Lagoon: Sludge	
#	Parasite	#/kg	#	Parasite	#/kg
1	Strongyles	2 000	1	Strongyles	2 000
2	Strongyles	1 000	2	Strongyles	1 000
	Toxocara	1 000			
3	Strongyles	3 000	3	Strongyles	2 000
	Toxocara	1 000			
4	Strongyles	1 000	4	No Parasites	
5	No Parasites		5	Strongyles	1 000
				Toxocara	1 000
6	Strongyles	3 000	6	Strongyles	3 000

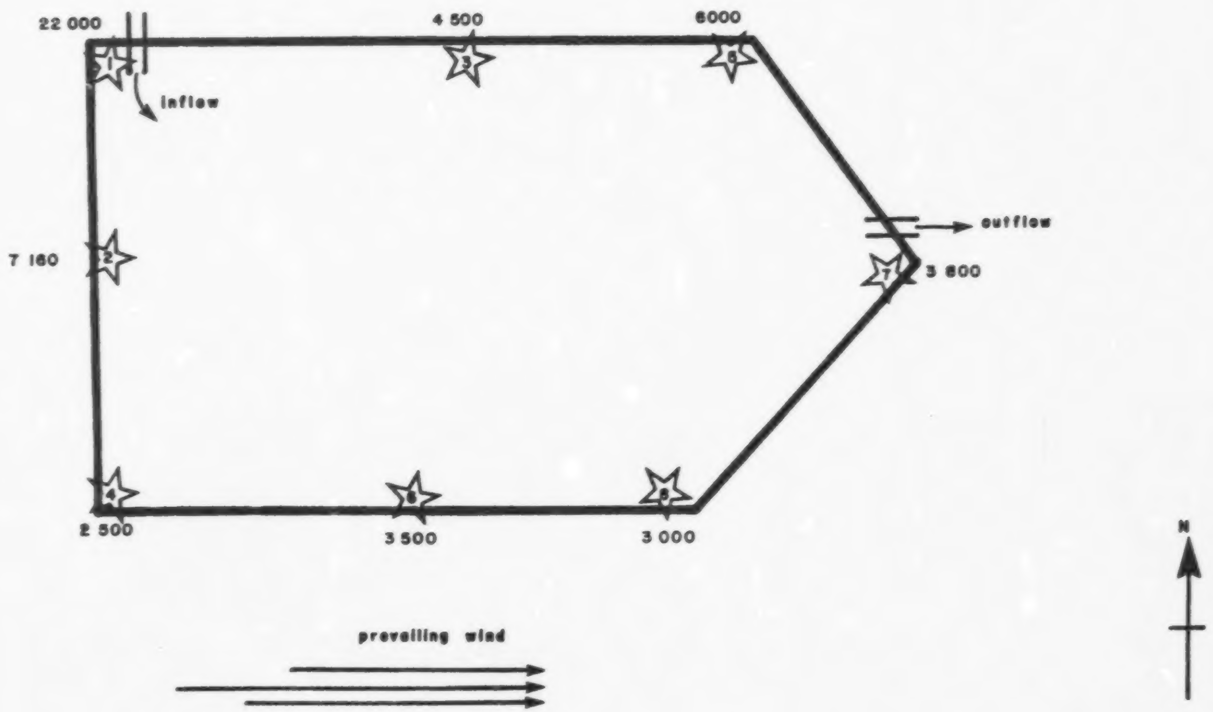
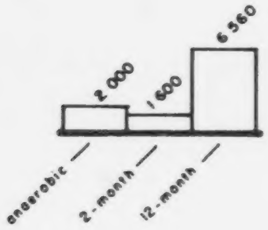


Figure 2. Crossfield: twelve-month lagoon sampling points and numbers of worm eggs per kg sludge.

Crossfield Lagoons:

average number of worm eggs per kg sludge



Crossfield 12-month Lagoon:

average number of worm eggs per kg sludge at eight sampling points

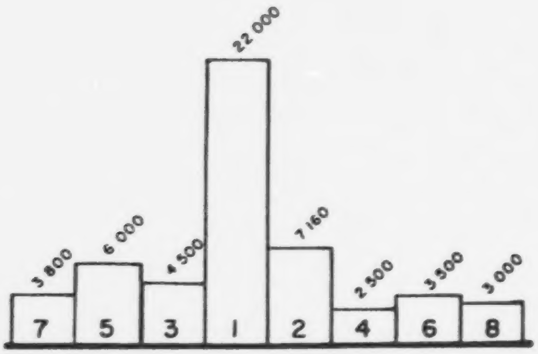


Figure 3. Crossfield lagoons.

lagoon constitutes a "dead end", the eggs would eventually settle out. The eggs may become re-suspended with wind and wave action and it is presumed that this factor accounts for the higher number of eggs along the north shore of the lagoon.

Sedimentation rates are known to be dependent upon the specific gravity of the bodies, with protozoan cysts being slowest (Cram 1943; Wang and Donlop 1954). Amongst the helminths, the eggs of *Taenia saginata* are among the last to settle out and could be wind dispersed (Liebmann 1964). *Ascaris* eggs settle most rapidly, followed by hookworm and *Trichuris* eggs (Bhaskarian et al. 1956).

The parasitic stages most frequently found in Crossfield appeared to be strongyle eggs of animal origin, and it is believed that the local source is a veterinary clinic treating both large and small mammals on the premises. The presence of predominately animal worm eggs was also found by Liebmann (1964) who reported that 90% of the one billion worm eggs discharged daily by major cities of Central Europe were of animal origin. Foster and Engelbrecht (1973) and Jackson et al. (1977) reflect this opinion.

5.1.2 Investigations at a Barnyard, Pasture, and a Spray Irrigated Field

Because of the predominance of animal parasites found in all of the sewage samples taken, it was considered important to take some additional samples from a barnyard and a pasture used for grazing cattle in order to gain some idea of normal conditions. Accordingly, arrangements were made with Mr. Cornish who owns a dairy farm adjacent to the Crossfield Lagoons. Mr. Cornish had also used some water from the 12-month holding lagoon to spray irrigate some additional grassland in 1979 so a small number of soil samples were taken from that field as well. All samples were taken in July and August of 1980.

The results from the barnyard and pasture inhabited by dairy farm cattle are reported in Table 7. The parasitological results in Table 7 show that the cattle from Mr. Cornish's barnyard are carrying strongyle nematodes. The pasture where these animals are turned out to graze appears to be only sparsely contaminated. This is presumably a consequence of the low probability of finding a patch of infected soil when a small number of animals is released

Table 7. Parasitological results from the barnyard and pasture.

Barnyard (Dairy Farm)			Pasture (Dairy Farm)		
Sample	Parasite	#/kg dry wt.	Sample	Parasite	#/kg dry wt.
1	Strongyles	6 000	1	Strongyles	1 000
2	Strongyles	28 000	2	Strongyles	none
3	Strongyles	14 000	3	Strongyles	none
4	Strongyles	4 000	4	Strongyles	none
5	Strongyles	9 000	5	Strongyles	none
6	Strongyles	1 000	6	Strongyles	none
7	Strongyles	8 000	7	Strongyles	none
8	Strongyles	20 000	8	Strongyles	none
9	Strongyles	23 000	9	Strongyles	none
10	Strongyles	2 000	10	Strongyles	none
11	Strongyles	2 000	11	Strongyles	none
12	Strongyles	8 000	12	Strongyles	2 000
13	Strongyles	3 000			
14	Strongyles	17 000			
15	Strongyles	4 000			
16	Strongyles	5 000			
17	Strongyles	20 000			
18	Strongyles	14 000			
19	Strongyles	7 000			
20	Strongyles	14 000			

into a large pasture. The animals in this barnyard and pasture appeared to be healthy and not suffering unduly from their parasite burden. It can only be assumed that this is the normal situation in the light of the large number of animal parasites found in sewage both at Crossfield and at Calgary and from later examination of animal faeces from a slaughterhouse. No parasites were found in four samples taken from the field sludged with lagoon effluent.

5.2 IDENTIFICATION OF EGGS FROM THE SLAUGHTERHOUSE AND SLUDGED FIELDS

Because domestic animals contribute considerable numbers of parasite stages to sewage and ultimately to sludged fields, it was considered essential to determine which parasites are found in slaughterhouse animals. In addition, larvae hatched from eggs obtained from the abbatoir were studied so that comparisons could be made with those found on sludged fields.

Initially, the procedure contemplated was to determine the adult parasites present in cattle, horses, and swine and then to continue with an analysis of egg morphology/size and larval characters following hatching. Trips to the slaughterhouse for parasite collection, and dissection of gastro-intestinal tracts at the Centre showed that such a procedure was untenable in terms of numbers of worms found and the time required for such examinations. Instead, faecal examinations of fresh faecal specimens taken from the slaughterhouse corrals were made for total numbers of eggs, possible identification according to the literature, and hatching experiments so that the relative numbers of larval species could be compared. Compilation of these data allow for the possible identification of eggs and larvae from sludged fields.

The tabular material following includes egg counts from the faeces of cattle, horses, and hogs from the slaughterhouse as well as the identification of larvae hatched from these eggs. Additionally, larvae recovered from sludged fields are noted as well as eggs and parasite stages which are infective to man. Table 8 summarizes the numbers and types of eggs found in faecal samples from the corrals. The term Strongyles is used as a collective term for the eggs of similar morphology of various gastro-intestinal nematodes from domestic animals. Table 9 summarizes the identification of larvae hatched from eggs from the same

Table 8. Eggs from the faeces of slaughterhouse animals (numbers/kg dry weight).

Host	Species	Positive/ Total	Mean	Range
Cattle	Strongyles	14/15	166 600	0 to 1 114 000
	Trichuris	1/15	713	0 to 10 700
Horses	Strongyles	15/15	317 700	3 250 to 980 000
	Parascaris	4/15	9 390	0 to 97 400
Hogs	Strongyles	11/15	39 700	0 to 475 000
	Ascaris	13/15	93 400	0 to 442 000
	Trichuris	1/15	625	0 to 9 360

Table 9. Infective Strongyle larvae hatched from slaughterhouse faeces.

Source	Parasite	#	% of sample
Cattle (composite of 5 animals)			
	Cooperia	(39)	45.3%
	Haemonchus	(16)	18.6%
	Trichostrongylus	(11)	12.8%
	Osteragia	(6)	6.9%
	Nematodirus	(5)	5.8%
	Chabertia	(4)	4.6%
	Oesophagostomum	(3)	3.5%
	Strongyloides	(2)	2.3%
Horses (composite of 5 animals)			
	Triondontophorus	(64)	60.4%
	Strongylus	(23)	21.7%
	Poteriostomum	(15)	14.1%
	Strongyloides	(3)	2.8%
	Trichostrongylus	(1)	0.9%
Hogs (composite of 5 animals			
	Strongyloides	(68)	78.2%
	Oesophagostomum	(19)	21.8%

faecal samples. The eggs of ascarids and Trichuris may be identified because of their distinctive morphology.

The data in Table 10 show that many of these Strongyle larvae also appear on the sludged fields. Ascarids and Trichuris spp. have also been found on the sludged fields but, as the eggs do not hatch before ingestion, the larvae were not found. Table 11 traces the occurrence of parasites believed capable of infecting man from the slaughterhouse to sludge to the sludged fields. The actual health hazard presented by these parasites has not yet been assessed but their presence on the sludged fields is confirmed.

5.3 THE SURVIVAL AND HATCHING OF PARASITIC NEMATODE EGGS IN VARIOUS MEDIA

The object of this experiment was to determine the viability, development, and hatching of the eggs of parasitic nematodes in faecal material, faeces mixed with sludged soil, and faeces mixed with lagoon sludge. A horse provided the source of parasite material. Fresh faeces were examined by flotation of eggs using the $NaNO_3$ (sp. gr. 1.25) method and it was noted that all of the eggs were alive and contained stages from the zygote through a series of blastomere numbers to morulae. Larvae were not seen either within the egg or in the free state.

Three samples were prepared from the above and placed in Petri dishes: (1) 30 g of horse faeces; (2) 20 g of faeces mixed with an equal weight of moistened aged (3 to 6 months), dry sludged field soil; and (3) 20 g of faeces mixed with an equal volume of raw lagoon sludge. This sludge contained only one to four presumed parasite eggs per g of wet sample. After smoothing the surface, the dishes were covered, placed in a closed chamber to prevent drying, and analysed at 1, 2, 4, 20, and 31 days. Analyses were made to determine the numbers and percentages of eggs in the early stages of development, eggs containing motile larvae, dead eggs, and free larvae. The results are reported in Figures 4, 5, and 6.

There are two factors to be considered in this study: survival of eggs under various conditions and the development of the surviving eggs. Viable eggs contained in their natural environment, manure alone (Figure 4), decreased in numbers by 60% over the 31 day period of the experiment. After 31 days, in excess of 97% of the living stages seen were in the form of infective

Table 10. Infective larvae found from sludged fields excluding Ascarids.

1980 Sludged Field

Spring sample, 1981: 5 positive, 25 negative samples (#/2.5 g wet wt.)

Trichostrongylus	3
Strongyloides	1
Cooperia	1

Summer sample, 1981: 19 positive, 54 negative samples (#/2.5 g wet wt.)

Trichostrongylus	9
Nematodirus	5
Haemonchus	4
Cooperia	4
Strongyloides	4
Oesophagostomum	3
Poteriostomum	3
Strongylus	2
Osteragia	2
Triodontophorus	1
Chabertia	1
Not identified	9

1979 Sludged Field

Spring sample, 1981: 2 positive, 28 negative samples (#/2.5 g wet wt.)

Nematodirus	1
Not identified	2

continued...

Table 10. Concluded.

Summer sample, 1981: 5 positive, 25 negative samples (#/2.5 g wet wt.)	
Osteragia	1
Chabertia	1
Not identified	3
1978 Sludged Field	
Spring sample, 1981: 2 positive, 28 negative samples (#/2.5 g wet wt.)	
Trichostrongylus	1
Not identified	1

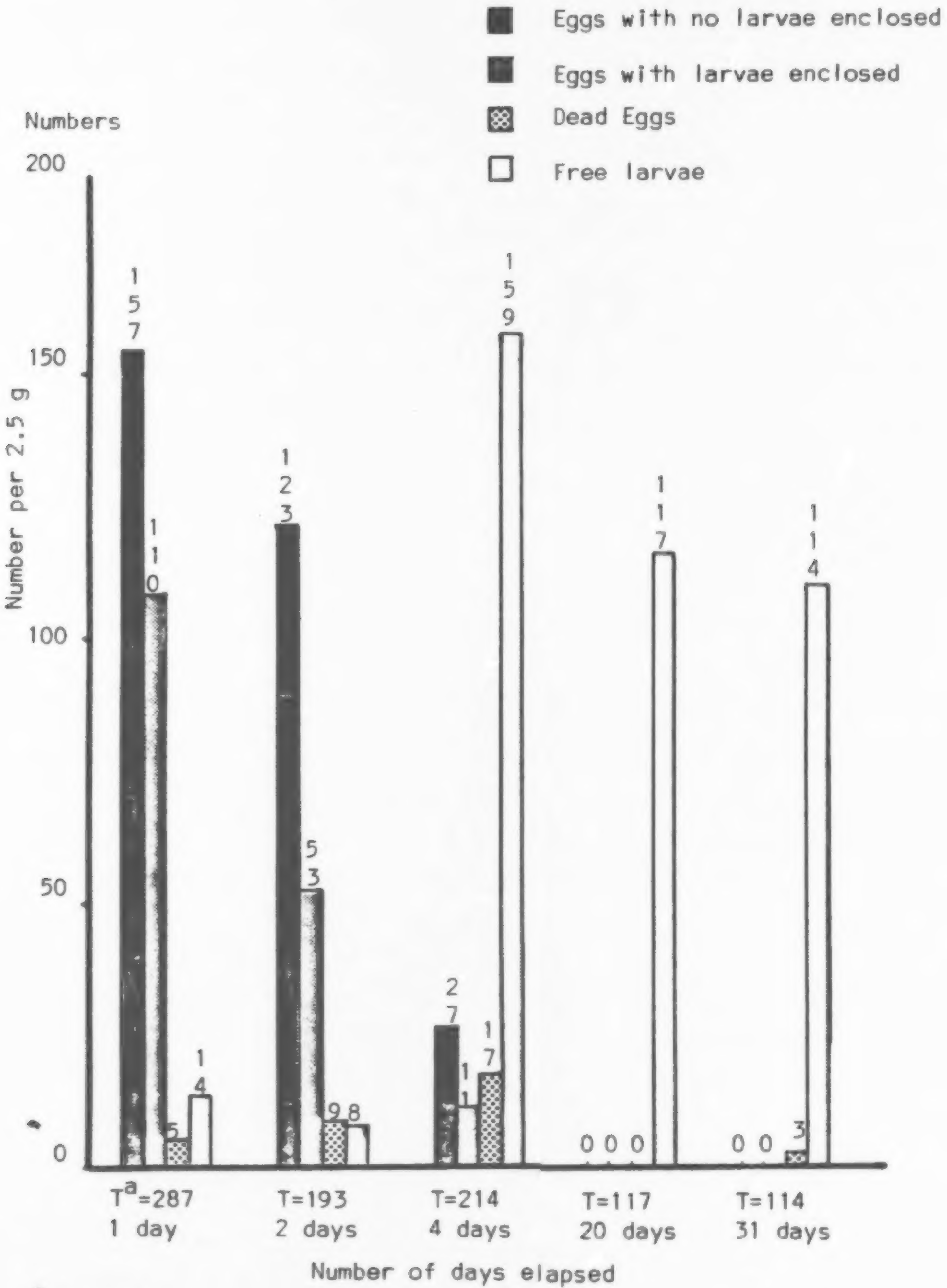
Table 11. Parasites found presumed capable of infecting man (according to literature sources).

Organism	Slaughterhouse	Lagoon Sludge	Sludged Land
Ascaris	E[a]	E	E, No free larvae
Enterobius		E	E, No free larvae
Haemonchus	E, L[b]	E?, L	E?, L
Neoascaris			E, No free larvae
Osteragia	E, L	E?	E?, L
Parascaris	E		
Toxocara			
canis		E	E, No free larvae
cati		E	E, No free larvae
Trichostrongylus	E, L	E?, L	E?, L
Trichuris	E	E	E, No free larvae
Toxascaris[c]		E	E, No free larvae
Oesophagostomum[c] (found in primates)	E, L	E?	E?, L

[a] E = Egg

[b] L = Larvae

[c] Could infect man?



T^a = Total

Figure 4. Horse parasite stages in manure. Numbers in vertical type.

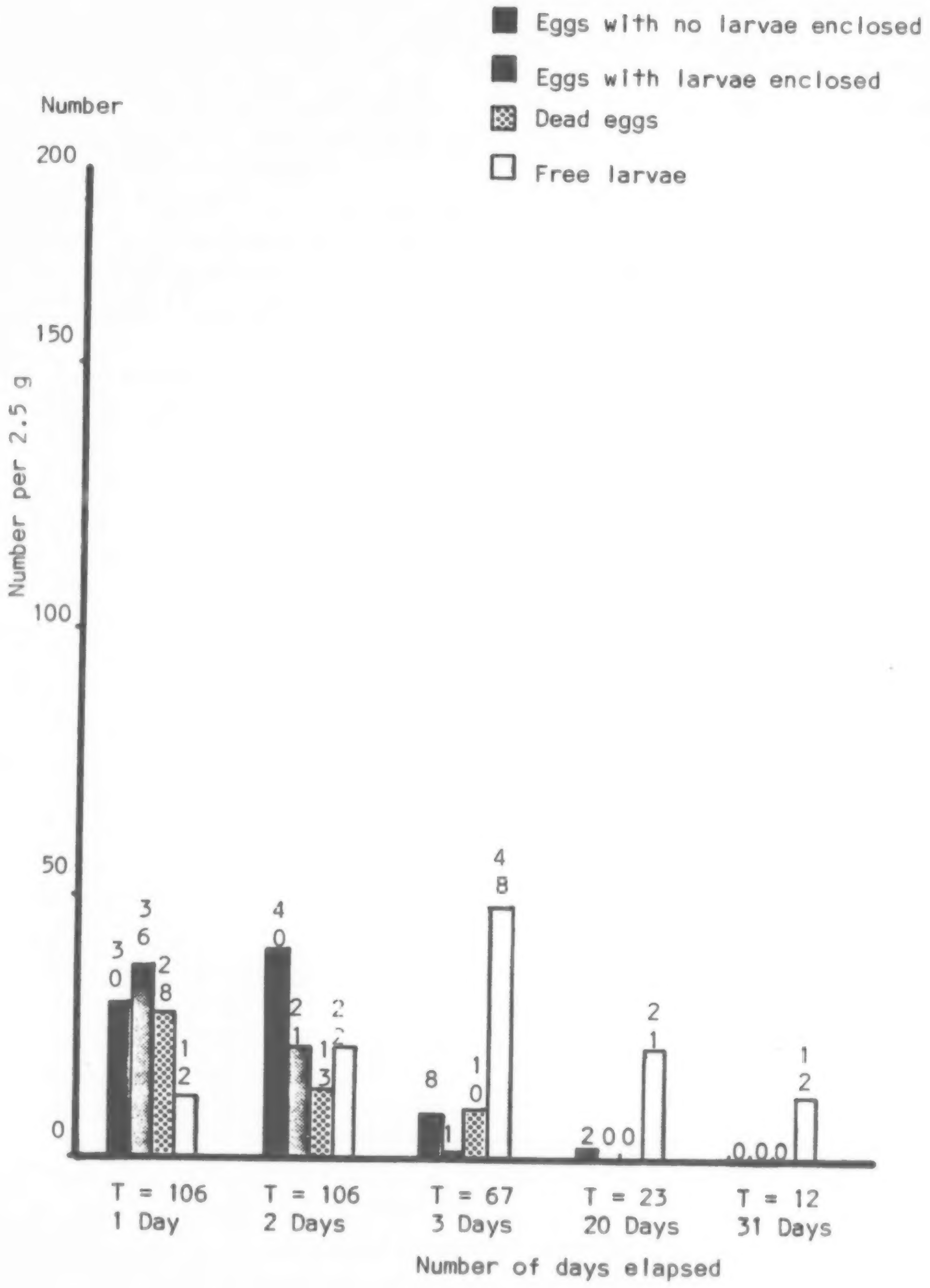


Figure 5. Horse parasite stages: manure mixed with sludged soil.

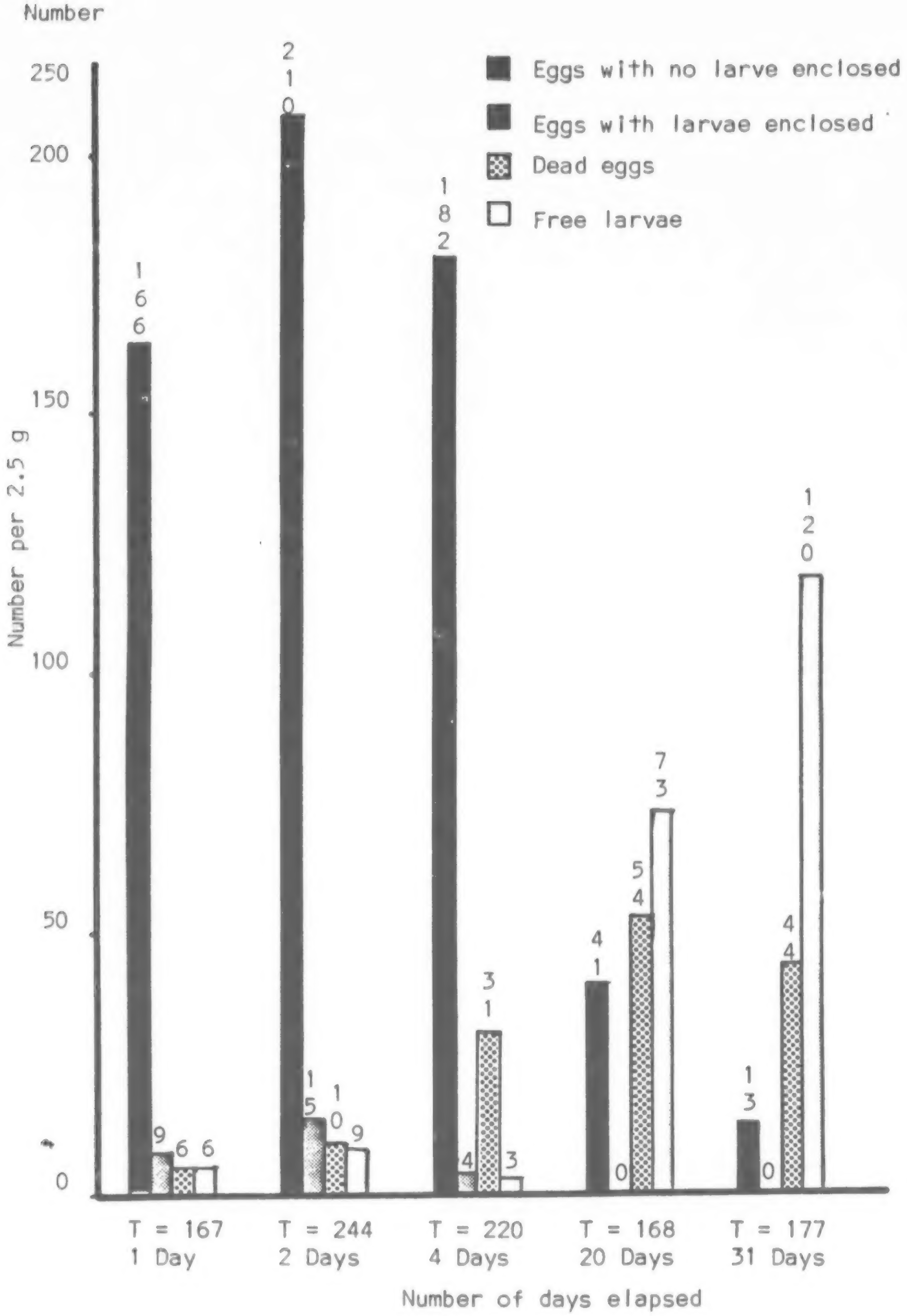


Figure 6. Horse parasite stages: manure mixed with lagoon sludge.

larvae. Nothing can be definitely stated about the cause of the demise of some 60% of the original inoculum except for the trite phrase "natural attrition". The World Health Organization (1967) suggests that the predatory activities of mites and soil fungi contribute to the low rates of survival of Ascaris eggs, but mites were not detected during these examinations (although they were seen in field samples) nor were fungi noted either in the samples or surrounding dead parasite stages. It may be presumed that autolysis and the activities of intrinsic manure bacteria accounted for the disappearance of the dead forms.

When manure was mixed with 1980 sludged soil, there was an 89% decrease of viable forms over the 31 day study period (Figure 5). All of the remaining living forms were infective larvae, indicating that the parasitic development was efficient insofar as end-product was concerned but inefficient regarding total numbers of infective larvae produced. Neither soil inhabiting mites nor fungi were seen in either the vicinity of or away from dead eggs although dead eggs were surrounded by masses of rod-shaped bacteria. Whether the bacteria were the cause of egg demise or simply opportunistic scavengers following death is not known, but it is interesting to note that no such activity was observed with dead eggs in the manure-egg mixture alone.

When eggs in manure were mixed with lagoon sludge, the results were considerably different (Figure 6). A survival rate of in excess of 94% of the parasitic stages over the 31 day period was observed and the numbers of viable, but undeveloped, eggs were higher than in the other experiments. Little (1980) believes that sludge provides ideal conditions for hatching, but the present investigation suggests that sludge may provide ideal conditions for static maintenance of eggs in the stages of early development. Hatched larvae are also protected in sludge. This view is also supported by the World Health Organization (1967) which found that eggs (Ascaris) stored for long periods of time in faecal deposit tanks (analogous to sludge) developed normally upon reaching soil. Inasmuch as eggs must be provided with oxygen to develop (Soulsby 1968) it seems probable that eggs contained within the anaerobic matrix of the sludge would remain in a delayed developmental state until such time as oxygen becomes available. In this experiment, oxygen became available at the surface of the mixture and, because of the moisture contained in the mass, the surface would also be moist and allow for hatching and subsequent development.

To speculate further, when sludge is deposited upon soil, eggs near the surface would remain in the early stages of development until such time as the presence of oxygen would allow for hatching. The moisture base evaporating outward from the inner mass of sludge would be adequate for both the development and the maintenance of the larvae through several stages. As drying progressed, larvae and eggs would die and, as aerobiasis progressed inwardly, more larvae would develop. The cycle would continue until the mass was sufficiently dry so as to destroy those eggs toward the center. Rainfall, irrigation, dew-fall, and ground cover as well as the thickness of the sludge deposit, however, would prolong the ultimate drying to death of static eggs. Few dead eggs were seen in the sludge-manure mixture and they were not surrounded by bacteria as they were in the manure-soil mixture. Neither mites nor fungi were noted.

The speculations noted above would prevail as long as the sludge remained integral on the surface of the soil and adequate moisture was present. Upon mixture with soil, it is conceivable that various parasitic stages would become exposed to those conditions which would result in decreased numbers of viable forms as in the manure-soil mixture. Presumably, this hostile environment would occur at the soil sludge interface and possibly would not affect the static eggs within the sludge masses. It should be noted that sludge upon drying becomes leathery but somewhat flexible and, within the larger pieces, viable eggs may be found. Sludge, therefore, can protect the eggs and delays the drying process, allowing for prolonged survival.

5.4 THE INFLUENCE OF DRYING ON WORM EGGS

As previously stated, one of the most significant causes of death among nematode eggs is dessication. In order to determine the effect of drying on eggs of parasitic worms, the following experiment was designed.

The source of nematode eggs was horse manure from an animal kept at the Kananaskis Centre. Parasite eggs were concentrated from this study by the process of slurrying 20 g of faeces with 200 mL H_2O, straining through cheesecloth, centrifuging and decanting of supernatant, and eventually reconstituting the mixture to a volume of 60 mL. Observation of the egg concentrate showed that all of the eggs were viable and contained only the early stages of development. None of the eggs contained larvae nor were free larvae at any stage noted. Twenty mL of egg concentrate were mixed with 60 g of aged horse faeces lacking parasites and 20 g of this mixture was put in a Petri dish. Secondly, 20 mL was mixed with an equal weight of moistened sterile (autoclaved), dry sludged soil from the 1980 sludged field. Finally, a similar mixture was made with fresh sludge from the Shepard lagoons. These samples were then smoothed and left, uncovered, on the benchtop. After 24 hours, samples of each mixture were removed. The percent moisture per mixture was determined by drying a subsample at 120°C for 24 hours and the numbers of developmental stages present in each sample were counted. At 48 hours, the analysis was repeated. After 72 hours, the samples were moistened and analysed after an additional 24 hours. The results are reported in Table 12.

The complete destruction of viable parasitic forms caused by drying is certainly not unexpected as moisture (in addition to proper temperature and the presence of oxygen) is necessary for not only the survival but the development of the eggs of parasitic nematodes (Soulsby 1968). The decrease of available water content (to 2.9 to 6.8% after 48 hours) was sufficient to invoke the demise of all forms. Free-living forms are exposed to the vagaries of climate and are typically able to re-colonize when conditions become suitable. Parasitic forms are more delicate, requiring critical temperature conditions for survival and hatching. The infective larval stage requires the reasonably rapid intervention of the definitive host before either being influenced by adverse environmental conditions or the depletion of food reserves.

Obviously, the conditions present in this short experiment did not duplicate the conditions found in sludged fields. In the latter, the quantities of sludge were far greater, allowing for greater water availability and retention. In addition, vegetation, debris, and large masses of sludge-soil mixtures can retain moisture and protect parasite stages. Prolonged drying periods in both warm and cold weather, however, would clearly cause significant reductions in parasite numbers.

Table 12. , Effects of drying on worm eggs[a].

Experimental Mixture	Numbers and % Eggs Viable Early Development	Numbers and % Eggs with Larvae	Numbers and % Dead Eggs	Numbers and % Free Larvae	Moisture Content %
24 Hours Drying					
Manure Alone	(0) 0	(8) 44.4	(10) 55.5	(0) 0	20.5
Manure & Soil	(15) 18	(46) 55.4	(22) 26.5	(0) 0	11.5
Manure & Sludge	(28) 65	(0) 0	(15) 34.8	(0) 0	11.8
48 Hours Drying					
Manure Alone	(0)	(0)	(16) 100	(0)	3.9
Manure & Soil	(0)	(0)	(18) 100	(0)	2.9
Manure & Sludge	(0)	(0)	(51) 100	(0)	6.8
24 Hours After Moistening 72 Hours Dried Sample					
Manure Alone	(0)	(0)	(19) 100	(0)	16.1
Manure & Soil	(0)	(0)	(25) 100	(0)	19.3
Manure & Sludge	(0)	(0)	(16) 100	(0)	22

[a] Numbers in brackets.

5.5 THE BONNYBROOK FACILITY: RAW SEWAGE AND DIGESTERS

In all cases strongyle-like eggs were the most common, with ascarid forms and other nematode species being present. The differentiation of strongyle species on the basis of eggs alone is a very difficult task because of their similar morphology, though overlapping size differences do occur.

The number of eggs found in raw sewage was found to average 7.4/L, with strongyle-like forms predominating followed by ascarids. Sludge from the anaerobic digester has appreciably more parasites (average of 3750/L) which can probably be accounted for by the sedimentation concentration of the solids in raw sewage. Aiba and Sudo (1964) found this when they reported 80 *Ascaris* eggs/L of raw sewage and 700 eggs/L in digested sludge. Graham (1981) found more than twice as many *Ascaris* in digested sludge as in raw sewage.

Much of the published data on the effect of the mesophilic anaerobic digestion process on animal parasites deals with *Ascaris*. Cram (1943), Kebina and Ploschcheva (1974), and Jolley and Fitzgerald (1976) all demonstrated that *Ascaris* eggs were little influenced by anaerobic digestion procedures, while Fitzgerald and Ashley (1977) noted that survival in digested sludge was dependent upon temperature. They report that exposure at 38°C for 21 days (about the temperature used at the Bonnybrook plant) killed all eggs under experimental conditions. Jolley and Fitzgerald (1976) also dealt with the eggs of the stomach nematodes of domestic animals (strongyles). They found that these organisms could survive anaerobic digestion of sludge at 39°C although hatched larvae were killed.

Various authors have found a variety of protozoan cysts and worm eggs in sludge lagoons including *Entamoeba*, *Eimeria*, *Isospora*, *Ascaris*, *Toxocara*, *Enterobius*, *Trichuris*, *Taenia*, and *Hymenolepis*. In Ontario, Graham (1981) found *Ascaris* (33 eggs/L liquid sludge), *Toxocara* (20 eggs/L liquid sludge) and, in addition, recorded *Trichuris*, *Toxascaris*, *Taenia*, and *Hymenolepis*. He states that anaerobically digested sludge has no effect upon these parasite stages. Fitzgerald and Ashley (1977) agree with this finding. That non-human worm eggs are so common is not surprising in view of the report of Liebmann (1964) who estimated that 90% of the 1 000 000 000 eggs placed in sewage every day by major Central European cities originated from animals. Foster and Engelbrecht (1973) and Jackson et al. (1977) concur on the importance of eggs of animal origin.

5.6 WORM EGGS RECOVERED FROM FIELDS SLUDGED IN 1978, 1979, AND 1980

Sludge known to contain parasites has been spread on a number of fields close to the Shepard Lagoon facilities over the past 12 years. An initial survey of the fields sludged in 1978, 1979, and 1980 was carried out during the earlier part of this study (in the spring and summer of 1980) to detect the presence of parasite eggs of sewage origin in the soil and to enumerate them. Subsequently, a more detailed sampling programme was commenced to describe the vertical and spatial (horizontal) distribution of these parasite eggs.

From fields sludged in 1978, 1979, and 1980, and lagoon sludge, single samples were taken on a number of occasions in the spring and summer of 1980 for parasitological (and microbiological) analyses. Eggs were extracted from soil samples using sucrose flotation during this part of the study.

A summation of the parasitological results from all field and sludge sources is reported in Figure 7. The results are reported in numbers per kg but the efficiency of the recovery of eggs using the sucrose measurement is only about 4%. These data indicate that the average number of eggs found per kg dry wt. drops steadily from a high of 37 600 eggs/kg dry wt. in lagoon sludge to a low of 1050 eggs/kg dry wt. in soil from the 1978 sludged field. The samples were taken from the soil surface and it was not certain whether the eggs had died or merely moved deeper into the soil.

5.6.1 Vertical Distribution of Worm Eggs: the 1980, 1979, and 1978 Sludged Fields

Because different periods of time had elapsed since sludging on these three fields, it was of interest to learn whether or not eggs had migrated downward in the soil or had simply disappeared. For this reason a series of 10 randomly selected samples was taken at three different depths in each of the fields. In this experiment, $NaNO_3$ was used as the flotation medium raising the egg recovery efficiency to 8%.

Shepard Sludged Fields:

average number of worm eggs per kg soil,
1978 - 1980

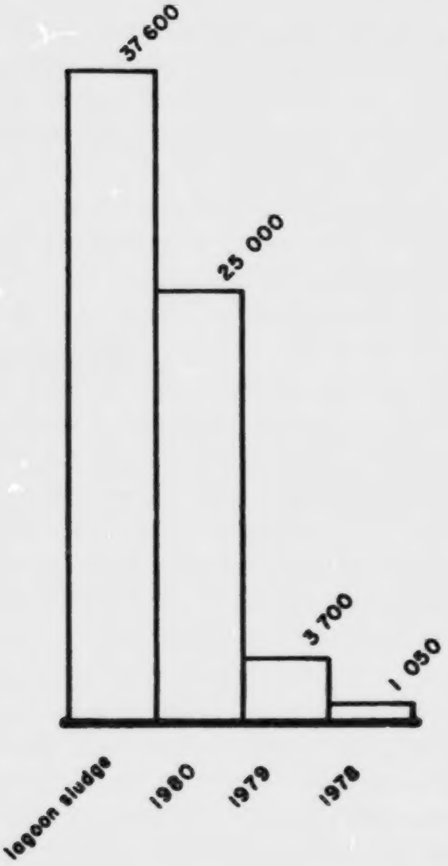


Figure 7. Average number of worm eggs in Shepard Lagoon Sludge and sludged fields.

The parasitological results are summarized in Table 13 and the following observations may be made. In the 1980 field, 85% of the eggs occurred in the top 2.5 cm with 13.4% in the 5 to 7.5 cm zone and only 1.6% on the lower hardpan. In fields sludged in previous years, there tends to be a lesser percentage of eggs in the upper layer and more in the middle layer, although even the 1978 field showed 68.5% of the eggs present in the top layer.

With regard to the vertical distribution of eggs in soil, Graham (1981) applied *Ascaris lumbricoides* var. *suum* to bare, well drained soil and recovered eggs up to 22 days later from the top 2 cm in 5 of 15 attempts. He found but one egg at a depth of more than 2 cm. This occurred two days after sludge application and he believed that this egg was carried to that location by the coring device. At 595 days, no eggs were found at any level. Graham (1981) concluded that there was little downward movement of eggs in well drained, bare soil. When the sludge and soil were mixed to a depth of 5 to 8 cm, eggs were found at the 2 to 4 cm level when 57 to 86 eggs/cm^3 were added with the sludge. They were not recovered more than 15 days after sludging-mixing. A report of the World Health Organization (1967) suggests that the low rate of survival of *Ascaris* eggs in lower levels of soil may be due to the predatory activities of mites and fungi. This mechanism has not been proven in the Shepard fields but the presence of mites was common in the soil samples. Although the numbers of eggs were shown to drop from 1980 to 1978 on fields sludged in those years, the data in Table 13 also suggest that some downward migration of eggs took place, presumably the result of discing. Even after two years, however, 68.5% of the eggs present occurred in the top 2.5 cm of soil. It should be remembered that the figures quoted in Table 13 are based upon the sodium nitrate method. Although this study demonstrated the superiority of that method over sucrose, its efficiency could not be raised more than 8%.

5.6.2 Spatial Distribution of Worm Eggs on the 1980 Sludged Field

In order to apply parametric statistical tests designed to prove differences between the populations found on the sludged fields, it is necessary to show that the data approximate a normal distribution. Accordingly, 40 sampling sites were randomly chosen

Table 13. Vertical distribution of parasite eggs on disced sludged fields.

			#/kg dry wt Depth			
	Upper (0 to 2.5 cm)	%	Middle (5 to 7.5 cm)	%	Lower (10 to 15 cm)	%
1980 Field: Total						
Mean	30 087	85	4 737	13.4	562	1.6
Standard Error	11 986		1 172		356	
1980 Field: Ascarids Only						
Mean	2 087	77.1	620	22.9	0	0
Standard Error	745		392		0	
1979 Field: Total						
Mean	39 162	79.7	9 412	19.2	551	1.1
Standard Error	23 207		2 030		348	
1979 Field: Ascarids Only						
Mean	1 974	56.8	1 231	35.4	273	7.8
Standard Error	570		650		260	
1978 Field: Total						
Mean	6 112	68.5	2 537	28.4	273	3.1
Standard Error	2 057		848		261	
1978 Field: Ascarids Only						
Mean	1 756	67.4	850	32.6	0	0
Standard Error	784		410		0	

(using grid co-ordinates and a random number table) on the 1980 field. Samples were taken from the top 2.5 cm only as previous work (see Vertical Distribution) indicated that 85% or more of the parasite eggs were found within this zone. The samples were analysed according to methods described earlier. The results are graphed in Figure 8.

These data show the eggs are not normally distributed. The distribution curve is leptokurtic and strongly skewed to the right. The mean is 4.95 eggs/g dry weight soil or 4950 eggs/kg and the range is from 0 to 82 eggs/g (13 200 eggs/kg), considerably larger than the mean. If the eggs are randomly distributed, then their distribution frequency should approximate the theoretical Poisson distribution. This hypothesis was tested with the raw data using a Chi-squared goodness-of-fit test but the hypothesis proved to be false. A binomial distribution does not apply because the assumptions upon which it is based were not valid for these data.

A natural logarithmic data transformation was then applied and the transformed data are graphed in Figure 9. The distribution of the transformed data resembled the theoretical normal distribution, but an application of the Kolmogorov-Smirnov goodness-of-fit test failed to substantiate this. The logged data were then tested for a Poisson distribution using the Chi-squared test again, and this time the null hypothesis could be accepted (x^2 = 4.32, χ^2_{crit} = 7.82). The transformed data therefore approximate a Poisson or random distribution. The fact that the untransformed data do not follow the Poisson distribution indicates that the eggs are not randomly spread about the fields. A close examination of the data in Figure 8 reveals that many clumps, or pockets, of eggs occur. This phenomenon may be observed even within a 200 g sample if repetitive subsamples are taken.

The pocketing effect is common in nature and this type of distribution is usually referred to as "contagious" (Shimwell 1971; Grieg-Smith 1964). Although individual eggs do occur in the soil, they more often occur as clumps or pockets for reasons discussed below. Because of this non-normal distribution, standard parametric tests such as t-tests cannot be applied to analysed the differences between fields. This has been demonstrated in the past and has been used as an explanation for uneven infection of tapeworms in cows (Crewe and Owen 1978). Graham (1981) found that

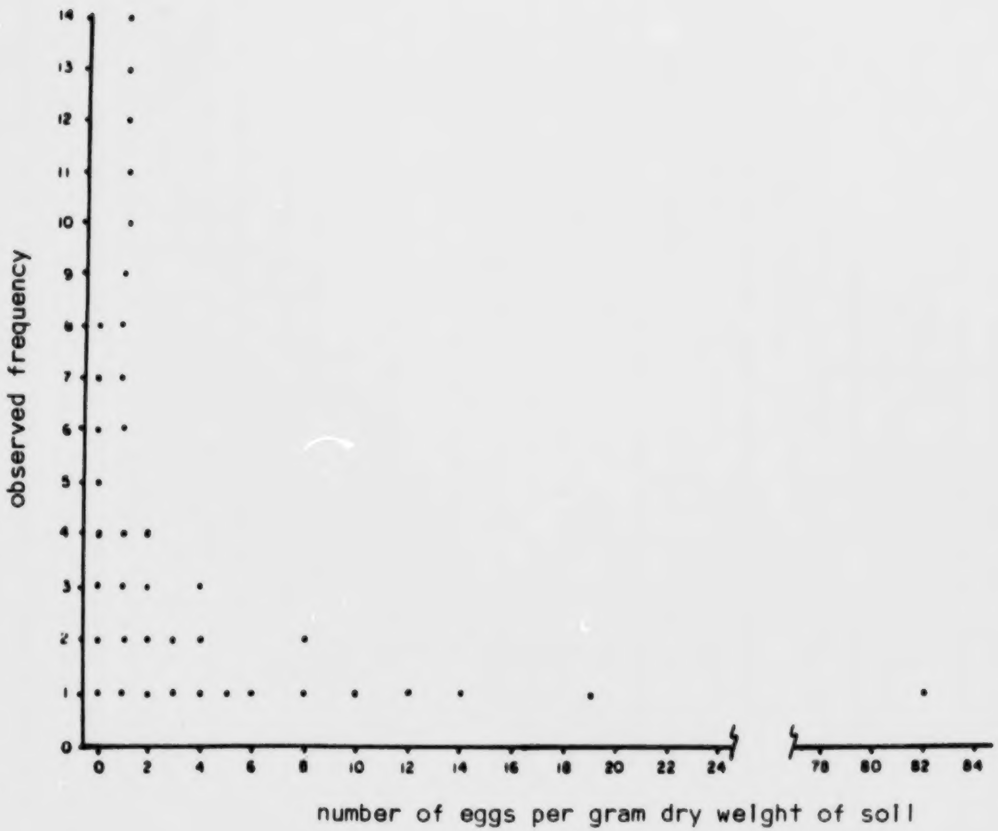


Figure 8. Distribution of parasite eggs on the 1980 sludged field (untransformed data).

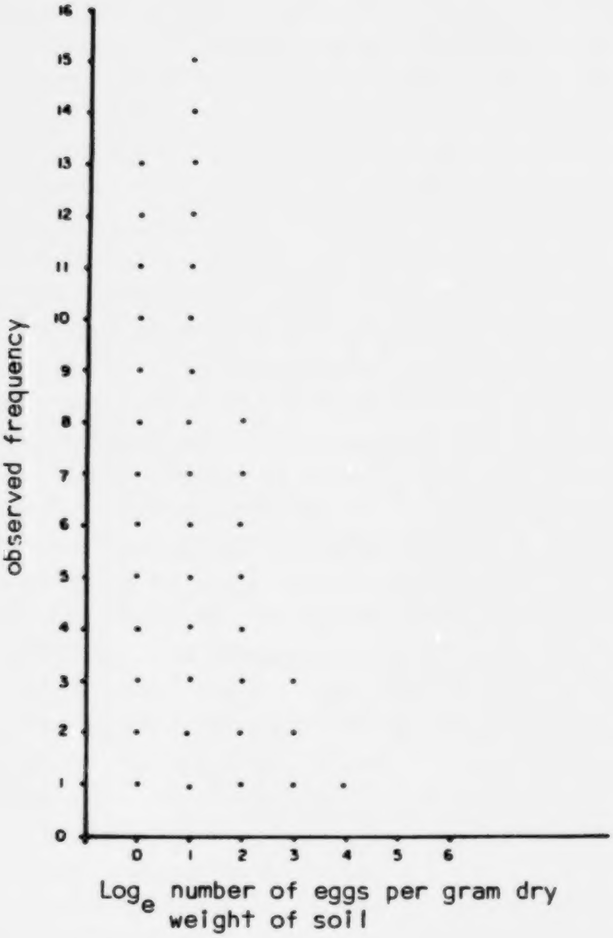


Figure 9. Distribution of parasite eggs on the 1980 sludged field (transformed data).

sludge applied to experimental plots could, because of irregularities in the surface, be spread unevenly on the soil surface. This results in thicker patches of sludge which might be expected to contain more eggs. He also made the following observations:

1. If there was a heavy rain before the sludge dried, the sludge would become more evenly distributed.
2. If the soil was dry upon application, the moisture from the sludge would quickly wet the underlying soil and there would be little lateral movement on level ground.
3. If the soil surface was already saturated before application, the sludge would flow rapidly into surface depressions resulting in an uneven distribution.

Graham (1981) also noted that sludge is thickened by settling in lagoons which could result in worm eggs having an uneven distribution. Beaver (1975) noted that soil particles can be moved some distance by heavy rainfall and, in addition, found that rain on unplanted soil can result in the concentration of nematode eggs under a layer of top soil. The World Health Organization (1967) found that, because of the sorting action of wind and rain on soil particles, *Ascaris* eggs are not randomly distributed. All of these mechanisms support the "pocketting" observation.

5.7 VIABILITY OF PARASITE EGGS ON THE 1978 TO 1981 SLUDGED FIELDS

Because of the results found in the immediately preceding section, fields sludged in 1978, 1979, 1980, and 1981 were then sampled quarterly (i.e., seasonally). Thirty samples selected randomly (using random numbers and a grid system) were taken on each sampling trip. The number of eggs in each sample varied widely within each set of thirty, ranging from zero to quite large numbers. As found in 1980, the data revealed that egg populations are not normally distributed. Indeed, unless the data are transformed to natural logarithms, they do not conform to any of the common frequency distributions (i.e., normal, poisson, binomial). If a natural logarithm transformation is performed, the data sometimes approximate a contagious poisson distribution.

Because of this variability, statistical comparison of the fields is made much more difficult. Fortunately, however, the relationship between egg survival and time is readily apparent and the large number of samples taken lends confidence to the results. Figure 10 is a graph of the arithmetic means and 95% confidence intervals calculated for all of the sample sets taken. The field from which each sample was taken is identified by the shape of the mean mark; otherwise, the only variables considered were time and egg numbers found per kg dry wt. of soil.

Inspection of Figure 10 reveals a rapid mortality of eggs over the first 18 months and very little change afterward. The mathematical relationship indicated is that of a negative exponential function. If a regression analysis is performed on the means, a correlation coefficient (r) of 0.83 is found. If all of the data are individually treated using a regression analysis, a much lower correlation coefficient is found ($r = 0.29$) because of the large variation in data. In order to compare a new field with the relationship reported in Figure 10, it would be necessary to randomly select 30 samples, analyse them according to our methods, and use the arithmetic mean in conjunction with the 95% confidence limit. The natural logarithms of the means have been plotted in Figure 11 to demonstrate a straight line relationship.

The predictive equation from Figure 11 may be easily applied to calculate the time required to reach such low levels of eggs that no more can be extracted using our methods (52 months). It is clear, however, that very low levels of eggs are reached within 18 to 20 months and the health hazard presented by parasite eggs is relatively slight after that time. This conclusion is complicated somewhat by the fact that areas of high egg concentration do exist but egg numbers drop rapidly over the first year.

5.8 THE 1981 FIELD BEFORE AND AFTER SLUDGING

The 1981 field was sludged in early May of that year. Prior to sludging, a regular series of 30 soil samples were taken from the surface of the field and analysed in the usual manner. No helminth eggs or larvae were found. The sludge which was applied to the field was also sampled and analysed and was found to contain 11 078±2913 eggs/kg dry weight. The following organisms, known to

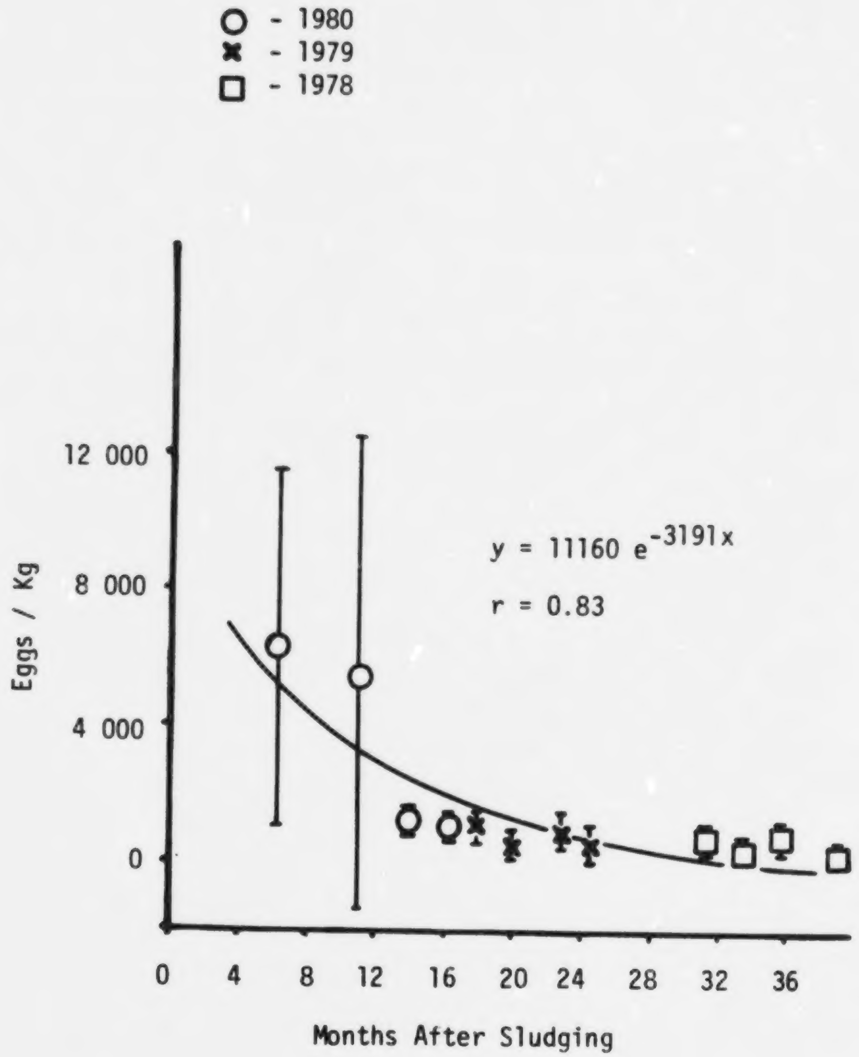


Figure 10. Survival of helminth eggs on sludged fields.

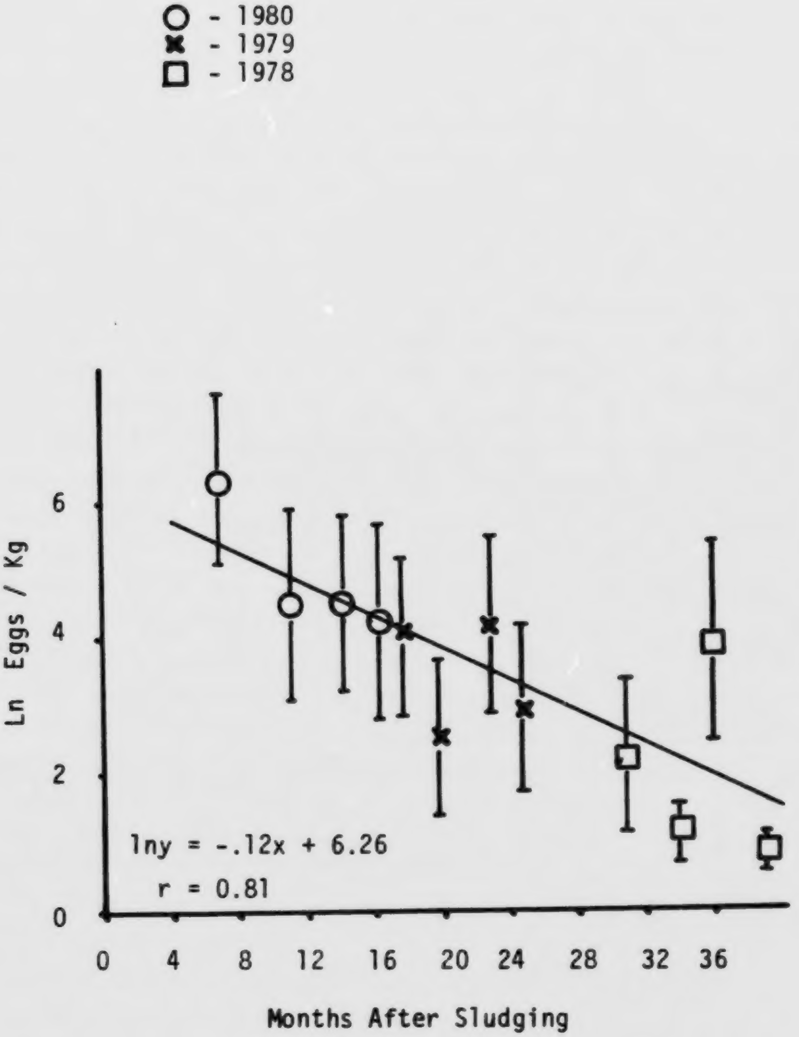


Figure 11. Survival of helminth eggs on sludged fields (data transformed to natural logarithms).

be capable of infecting man, and which could be identified, were found in the sludge: *Ascaris*, *Enterobius*, *Toxocara canis*, *Toxocara cati*, *Toxascaris*, *Trichuris*. Before the field was disced, two samples of sludge from the surface of the soil were taken and five subsamples of each were analysed. The results showed that 7244±2960 eggs/kg were still viable one week after sludging. The field was disced at the end of May and allowed to lie fallow for the remainder of the year.

Four months after sludging, 15 soil samples were taken and no viable eggs were found. Thirty more samples were taken six months after sludging with similar results. No eggs or larvae considered to be parasitic were found during any of the eight 1982 samplings, winter, spring, summer and fall.

Fifteen samples from a depth of 10 to 15 cm were taken four months and six months after sludging and showed 770 and 574 eggs/dry weight kg, respectively. Fifteen samples taken from this depth during the spring of 1982 (11 months after sludging) revealed no eggs.

The results from the 1981 field are in direct contrast with those found the preceding year for the 1980 field. The fields are adjacent and similar in every respect, except that the 1980 field was planted (with rapeseed) very soon after sludging while the 1981 field lay fallow all summer. Although weather records were not kept at the field sites, the summer was average with numerous hot days. Presumably, the lack of vegetation cover permitted more rapid drying and direct exposure to sunlight with consequent increase of temperature which caused a high rate of egg mortality. This is consistent with other data presented in the literature as discussed below.

Surface temperatures in bare soil can be quite high when exposed to strong sunlight. Graham (1981), in Ontario, found that surface temperatures could be as high as 47°C on the surface but not over 26°C at a depth of 8 cm. Levine (1968) found even higher temperatures (60°C) in Illinois soils and equally high temperatures in exposed sheep pellets. If vegetation cover is present, soils would be partially protected and moisture content would be higher.

Other climatological factors, in addition to temperature, which influence the longevity of parasite stages are direct sunlight and soil moisture. The World Health Organization (1967) states that Ascaris eggs on moist sand are killed by direct sunlight but found that if yellow or green filters were used the eggs would survive. The World Health Organization (1967) also studied the effect of soil moisture content upon survival of Ascaris and found that when soil moisture reached a minimum of 4% all eggs were killed within 4.5 hours.

When dealing with sludged fallow land (the 1981 field) and the data noted above, it would appear that a combination of sunlight, temperature, and dessication contributes to the demise of worm eggs. Soils at the Calgary fields could conceivably reach the same temperatures observed by Graham (1981) and sludge might be considered analogous to the sheep pellet data reported by Levine (1968). The percentage of moisture in the top 2 cm of the 1981 field averaged 4.78% in August, which corresponds to the lethal humidity noted by the World Health Organization (1967); direct sunlight would influence only those parasite stages which were at the surface.

5.9 THE 1982 SLUDGED FIELD

The fact that the field sludged in 1981 showed a rapid disappearance of parasite stages suggested that summer fallowing led to a destruction of parasites, presumably by dessication, elevated temperature, and sunlight. This field was disced, however, and a few parasite stages were transferred to the subsurface as mentioned above.

It was reasoned that if after sludge application discing was not allowed there would be no mechanical displacement of parasite stages to the subsurface. All parasites would then be exposed to environmental extremes in the 2 cm or so of the soil surface during the period of summer fallow with a resulting complete kill.

At this point, however, theory and practice separated. We were informed that summer fallowing of sludged, undisced land was impractical because exposure of surface sludge resulted in the loss of valuable ammonia. In addition, surface exposed sludge dried rapidly and became too leathery to easily mix with the soil by discing, and applied but undisced sludge would allow for the rapid growth of weeds. Farmers agreed and would not allow sludging without subsequent discing. We were able, however, to obtain the co-operation of the City of Calgary which laid sludge over grass in a small plot of approximately 5 x 20 m at the Shepard site. Even though the method would presumably never be employed, it seemed reasonable to pursue the trial.

This plot was analysed seven days after sludge deposition and 7000 parasite eggs/dry weight kg were found. No parasite eggs were found in subsequent examinations (after 43 and 83 days).

5.10 THE PASTURE

This field, grazed by cattle, showed parasite eggs at every analysis. The winter samplings (February and March) revealed 219 and 162 eggs/ kg dry wt. During spring (May), 221 and 933 eggs were seen while the summer analyses (June and July) showed 826 and 400 eggs/kg dry wt. Fall analyses (September and October) revealed 458 and 589 eggs/kg dry wt.

The pasture and the control fields were adjacent and presumably deer co-existed with the cattle and could have contributed parasite stages. Other wild animals could have likewise been responsible for some of the parasite stages. To demonstrate that cattle were contributing parasite stages, three manure samples were examined in May 1982. The eggs found numbered 4375, 71 000 and 91 000/kg dry wt.

5.11 THE CONTROL FIELD

This field, planted in hay and ungrazed for a minimum of 3 years, was selected to provide baseline data for sludged fields with regard to the disappearance of potential health hazards.

Winter samples (January and March) of 1982 failed to show parasite eggs. During spring sampling (May and June), eggs presumed to be of parasite origin were found in the amounts of 95 and 319/kg dry wt. The summer sampling periods (July) displayed 515 and 262 eggs/kg dry wt. while fall analyses (September and October) showed 217 and 162 respectively.

Although a control field had not been sludged or exposed to grazing domestic animals, scattered deer droppings were found. It was assumed that parasite stages could be attributed to this source and, presumably, other small mammals and birds could have been contributory. A single examination of deer droppings revealed in excess of 2200 eggs/kg dry wt.

5.12 RAW, ANAEROBIC DIGESTER, AND LAGOON SLUDGE

Because the sludge to be applied to agricultural land in future years will not be aged so long in the storage lagoons, some additional samples were taken from the Bonnybrook and Fish Creek Sewage Treatment Plants in April 1982. The parasitological results are reported in Table 14. Two samples of raw and mesophilically digested sludge from each treatment plant were analysed. The figure for lagoon sludge is the average of 23 samples.

The numbers of eggs recovered from both digesters tend to be similar and the numbers of eggs found from lagoon sludge are comparable to the numbers from the digesters. Sludge appears to have the ability to maintain eggs in a static condition as shown by laboratory experiments discussed in this report. Eggs were shown to be concentrated in the digester sludge compared with the raw sludge presumably because of the longer retention time at this stage and the increased potential for settling.

5.13 BURIED/INJECTED SLUDGE

Two examples of subsurface sludge were investigated. In one instance, lagoon sludge was buried by hand 20 cm below the surface of unsludged land. In the other, sludge was machine injected 15 cm into land, disced, allowed to summer fallow, again disced,and then planted with winter rye.

Sludge hand buried in September 1981 contained 17 500 eggs/kg dry wt. Two hundred and fifty-eight days later, the number of viable, presumed parasite eggs had dropped approximately two orders of magnitude to 221/kg dry wt. Later attempts to find the sludge (277 and 313 days) were futile.

Machine injected sludge was examined 7 days after placement (June) and 10 900 eggs/kg dry wt. were found. Sixty-six days after injection and 55 days after the first discing, no eggs were found from the top 2.5 cm samples. Again, no eggs were found 91 days after injection and 16 days following the second discing.

Table 14. Parasite eggs found in raw, digested, and lagoon sludge in April 1982.[a]

Raw Sludge Fish Creek		Digester Sludge Fish Creek		Digester Sludge Bonnybrook		Lagoon Sludge
#1	#2	#1	#2	#1	#2	Av. of 23
2000	2976	10 500	10 710	10 740	15 150	11 078

[a] (eggs/kg dry wt.).

6. RESULTS AND DISCUSSION: BACTERIA

6.1 CROSSFIELD

6.1.1 The Crossfield Sewage Lagoons: Faecal Coliforms and *Salmonella*

The faecal coliform results for the liquid fractions sampled from the Crossfield sewage lagoons are reported in Table 15. From these it can be seen that the concentrations of the organisms declined steadily as water progressed through the various lagoons. This system appears to function very effectively in reducing the number of faecal coliforms. No significant temporal differences in the number of faecal coliforms were observed throughout the course of our sampling routine. Five *Salmonella* isolates were found in samples from the first anaerobic pond but none were recovered from either the two-month or 12-month lagoons.

Bacteriological analyses were also made from the bottom sludge samples collected around the perimeter of the 12-month lagoon. The results are reported in Table 16 and Figure 12. These results suggest that the distribution of faecal coliforms is more uniform in the 12-month lagoon sediments than the parasite eggs.

Bacteriologically, the lagooning system at Crossfield appears to be efficient with respect to the reduction in faecal coliforms. These diminished dramatically from the intake at the anaerobic pond, through the 2-month holding lagoon, and thence into the 12-month lagoon where an MPN of only 20/100 mL was recorded. *Salmonella* spp. were recovered on five occasions from the intake anaerobic pond, but not from either the two or 12-month lagoons. The above information corresponds to data reported by other workers. Kabler (1959), quoting Fitzgerald and Rohlich, noted that lagooning reduced bacterial numbers in general by more than 99% and that *Salmonella typhi* were completely destroyed. *E. coli* were reduced by 90% and Kabler felt that reduction of all enteric bacteria would follow the trend of *E. coli*. Similar reports for reduction of both coliforms as well as pathogens have been made by Cody and Tischer (1973) and Kott (1973). Bell (1976) found that the population of *Salmonella* was usually only 0.1% of faecal coliforms, and that a residual MPN of 13 *Salmonella*/100 mL did not pose a threat to livestock eating effluent irrigated forage.

Table 15. Crossfield lagoon system: faecal coliform analysis.

Date	Anaerobic Pond			2-Month Lagoon			12-Month Lagoon		
		95% Confidence Limits			95% Confidence Limits			95% Con. Limits	
	MPN/100 mL	Lower	Upper	MPN/100 mL	Lower	Upper	MPN/100 mL	Lower	Upper
3 June 1980	3.3×10^6	1.1×10^6	9.3×10^6	$\geq 2.4 \times 10^4$			50	<5	130
8 July 1980	3.5×10^7	1.2×10^7	1.0×10^8	2.0×10^3	<50	7.0×10^3	20	<5	70
29 July 1980	1.7×10^7	4.3×10^6	4.9×10^7	5.0×10^2	<50	1.3×10^4	20	<5	70
26 Aug. 1980	5.4×10^7	1.8×10^7	1.4×10^8	1.1×10^3	2.0×10^2	2.5×10^3	20	<5	70
2 Sept 1980	2.4×10^7	6.8×10^6	7.5×10^7	9.2×10^3	3.0×10^3	3.2×10^4	20	<5	70

Table 16. Results of the bacteriological analysis of bottom sludge around the 12-month lagoon (cells/100 mL).

Crossfield Sample Location	Sludge From 12-Month Lagoon Faecal Coliforms (MPN)		
		95% Confidence Limits	
		Lower	Upper
#1 - Inlet pipe	2.0×10^2	7.0×10^2	50
#2 - 50 yds. south of inlet pipe	1.40×10^3	4.0×10^2	3.4×10^3
#3 - 50 yds. east of inlet pipe	2.20×10^3	7.0×10^2	6.7×10^3
#4 - SW corner of lagoon	5.0×10^2	<50	1.3×10^3
#5 - NE corner of lagoon	1.1×10^3	2.0×10^2	2.5×10^3
#6 - midway between #4 and #8	5.0×10^2	<50	1.3×10^3
#7 - near outlet pipe	1.7×10^3	5.0×10^2	4.6×10^3
#8 - SE corner of lagoon	$<2.0 \times 10^2$		

Table 15. Crossfield lagoon system: faecal coliform analysis.

Date	Anaerobic Pond			2-Month Lagoon			12-Month Lagoon		
		95% Confidence Limits			95% Confidence Limits			95% Con. Limits	
	MPN/100 mL	Lower	Upper	MPN/100 mL	Lower	Upper	MPN/100 mL	Lower	Upper
3 June 1980	3.3×10^6	1.1×10^6	9.3×10^6	$\geq 2.4 \times 10^4$			50	<5	130
8 July 1980	3.5×10^7	1.2×10^7	1.0×10^8	2.0×10^3	<50	7.0×10^3	20	<5	70
29 July 1980	1.7×10^7	4.3×10^6	4.9×10^7	5.0×10^2	<50	1.3×10^4	20	<5	70
26 Aug. 1980	5.4×10^7	1.8×10^7	1.4×10^8	1.1×10^3	2.0×10^2	2.5×10^3	20	<5	70
2 Sept 1980	2.4×10^7	6.8×10^6	7.5×10^7	9.2×10^3	3.0×10^3	3.2×10^4	20	<5	70

Table 16. Results of the bacteriological analysis of bottom sludge around the 12-month lagoon (cells/100 mL).

Crossfield Sample Location	Sludge From 12-Month Lagoon Faecal Coliforms (MPN)	95% Confidence Limits Lower	Upper
#1 - Inlet pipe	2.0×10^2	7.0×10^2	50
#2 - 50 yds. south of inlet pipe	1.40×10^3	4.0×10^2	3.4×10^3
#3 - 50 yds. east of inlet pipe	2.20×10^3	7.0×10^2	6.7×10^3
#4 - SW corner of lagoon	5.0×10^2	<50	1.3×10^3
#5 - NE corner of lagoon	1.1×10^3	2.0×10^2	2.5×10^3
#6 - midway between #4 and #8	5.0×10^2	<50	1.3×10^3
#7 - near outlet pipe	1.7×10^3	5.0×10^2	4.6×10^3
#8 - SE corner of lagoon	$<2.0 \times 10^2$		

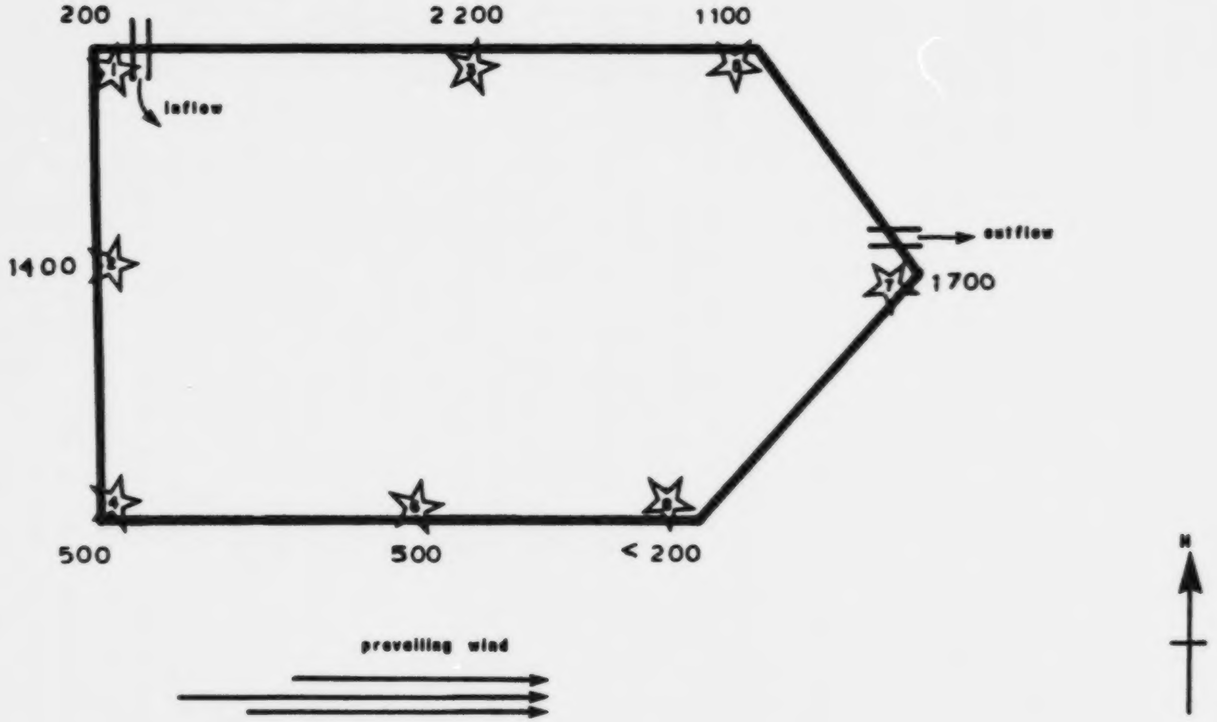


Figure 12. Crossfield: twelve-month lagoon sampling points and faecal coliform numbers per 100 mL sludge.

6.1.2 The Barnyard and Pasture near Crossfield

The microbiological data (Table 17) show that faecal coliforms are present in the barnyard in large numbers. The results were much lower in the pasture but their presence was still detected. No *Salmonella* were found at any time in either place.

Faecal coliforms were not found on the grassland (not used for grazing) spray irrigated in 1979 from the 12-month lagoon. This not surprising in the light of the parasitological and bacteriological data for the liquid fraction of the 12-month lagoon. There appears to be little hazard associated with the use of this water for spray irrigation.

6.2 THE SLUDGED FIELDS, CONTROL FIELD, AND PASTURE

6.2.1 The Faecal Coliforms

As noted in Table 18, the presence of faecal coliforms seems to be erratic in that, in some instances, the organisms could be found on fields after sludging, would disappear from analytical view during the winter, and reappear during the following warmer months.

The method employed for faecal coliforms involved determining those organisms capable of producing gas from lauryl tryptose broth and, upon transfer, being able to produce gas in EC broth at 44.5±0.2°C. The generally accepted organism capable of this reaction is *Escherichia coli*, though Dufour (1977) indicates that *Klebsiella* can accomplish the same. Dufour (1977) states that other Enterobacteriaceae are also capable of responding positively to the EC test and Leclerc et al. (1977) have found that certain strains of *Citrobacter* and *Enterobacter* can grow in EC at 44.5±0.2°C.

The data reported in Table 18 are enigmatic and controversy exists. Kenner et al. (1971) and Trout et al. (1975) were unable to find faecal coliforms from normal soil. Hinsley et al. (1972) indicated that *E. coli* initially grows in soil with a 5% moisture content but that by 30 days the number was reduced by 99%. Miller (1974) confirmed this initial growth followed by a decline of numbers and noted that low numbers were found after the six month interval. Kenner et al. (1971) found faecal coliforms on land sludged with anaerobically digested sludge after 21 weeks. The

Table 17. Crossfield: faecal coliforms.

Date		Barnyard			Grazed Pasture	
		95% Confidence Limits			95% Confidence Limits	
	MPN/100 g	Lower	Upper	MPN/100 g	Lower	Upper
3 June 1980	$\geq 2.4 \times 10^5$					
29 July 1980	1.3×10^6	3.0×10^5	3.1×10^6	2.0×10^3		
26 Aug. 1980	$\geq 2.4 \times 10^6$			9.4×10^4	2.8×10^4	2.2×10^5
2 Sept 1980	8.0×10^5	1.0×10^5	1.0×10^6	$<2.0 \times 10^3$		

Table 18. Faecal coliforms isolated by MPN.

	1978 Field	1979 Field	1980 Field	1981 Field	Control Field	Pasture	1981 Field
Spring/Summer 1980	+ (3/3)	+ (3/3)	+ (3/3)				
Winter 1980 to 1981	- (0/2)	- (0/2)	- (0/1)				
Spring 1981	- (0.2)	- (0.2)	- (0/3)				
Summer 1981	+ (1/2)	+ (2/2)	- (0/3)				
Fall 1981	+ (1/2)	+ (1/2)	+ (2/3)	+ (1/3)			
Winter 1981 to 1982			- (0/1)	- (0/1)		- (0/1)	- (0/1)
Spring 1982	- (0/1)	- (0/1)	+ (1/2)	+ (1/2)		+ (2/2)	- (0/2)
Summer 1982	- (0/1)	- (0/1)	- (0/2)	- (0/2)	+ (1/1)	+ (1/2)	+ (1/2)
Fall 1982			- (0/2)	- (0/2)	- (0/2)	+ (1/2)	- (0/2)

above indicates that faecal coliforms are not to be found from unadulterated soil but that, when land is treated with material containing faecal coliforms, there can be a residue for up to six months though no absolute die-off time is apparent.

Contrary to the above, Cliver (1980) believes that E. coli persists in sludge of all ages and Levin (1977) feels that there is the possibility of coliforms multiplying under natural conditions. Edmonds (1976) reported that faecal coliforms, applied with sludge, disappeared analytically after the advent of winter (267 days) but that there was an aftergrowth during warmer weather. Edmonds also implied that, contrary to the findings of Kenner et al. (1971) and Trout et al. (1975), faecal coliforms populated normal soil. Regarding colonization of soil by the Enterobacteriaceae, Gerba et al. (1975) reported that Salmonella spp. are capable of reproduction in soil during warmer months. The possibility also exists that the contamination of soil (or sludged soil) can occur as the result of the presence of wild animals which inhabit the area, either permanently or transiently. This aspect has been addressed by Cliver (1980) and Prestwood (1980) who believe that these animals can act as reservoirs of pathogenic agents. The distinct possibility exists, however, that in addition to carrying pathogens and indicators away from fields, such animals may also contribute to the pathogenic and non-pathogenic microbial population of the field.

In other circumstances, an additional possibility for invasion, or re-invasion, of soil by faecal coliforms is from buried sludge when it is manipulated to the surface during discing. The work of Temple et al. (1980) showed that there were 10^3 to 10^4 E. coli remaining eight weeks following the burying of human faeces in normal soil Trout et al. (1975) found almost complete reduction of Escherichia and Klebsiella buried with sludge after 2.5 months. In both instances, reduction of the organism but not eradication occurred. The above presents a feasible method for the observed re-contamination of surface soil with E. coli in this study. Eight months after burying sludge (September to May) faecal coliforms were isolated by MPN and E. coli was identified. Discing could bring the organisms to the surface for subsequent recontamination.

Regarding the presence of faecal coliforms from the pasture, we can assume that recurrent contamination by resident cattle is the source of the faecal coliforms. The isolation of the organisms from the control field, which is transiently occupied by deer (and presumably other vertebrate carriers), may be presumed to be the result of faecal contamination by these animals. The possibility that faecal coliforms populate normal soil or that they are capable of growth in soil does exist.

6.2.2 The Total Coliforms

The coliform group of bacteria constitutes an important assemblage with reference to faecal contamination, and potential health hazards, of sludged fields. The identification of members of the coliform group and their percentages of total number of isolates yields important data regarding the similarity between sludged fields and the control field (theoretically, no health hazard), and the dissimilarity between the pasture and the control field.

Dufour (1977) states that the total coliforms include several genera (*Escherichia*, *Citrobacter*, and *Enterobacter*) " some of which are seldom associated with faecal contamination." Dufour (1977) contends that *E. coli* is the only undoubted inhabitant of the human gut and that *Klebsiella*, *Enterobacter*, and *Citrobacter* can be isolated from human faeces in but small numbers. Leclerc et al. (1977) noted a departure from the above: *E. coli* was consistently present from human faeces. *Citrobacter* was noted in 66% of 30 samples while *Klebsiella* and *Enterobacter* were recorded 50% and 10%, respectively. The 14th edition of Standard Methods for the Examination of Water and Wastewater (Rand et al. 1976) further restricts the coliforms to *E. coli*, *Citrobacter freundii*, and *Enterobacter aerogenes* which in effect limits the coliforms to those species which can be isolated routinely, or less frequently, from faeces. Their presence in the soil implies faecal contamination and potential health hazard.

An examination of Figure 13 shows the combined percentages of the three species designated as total coliforms as found from the 1980 and 1981 sludged fields, the pasture, and the control field. These data show that the sludged fields are indistinguishable from the control field, while the pasture contains much higher percentages of these three species. These data suggest that the sludged fields present less health hazard than the pasture.

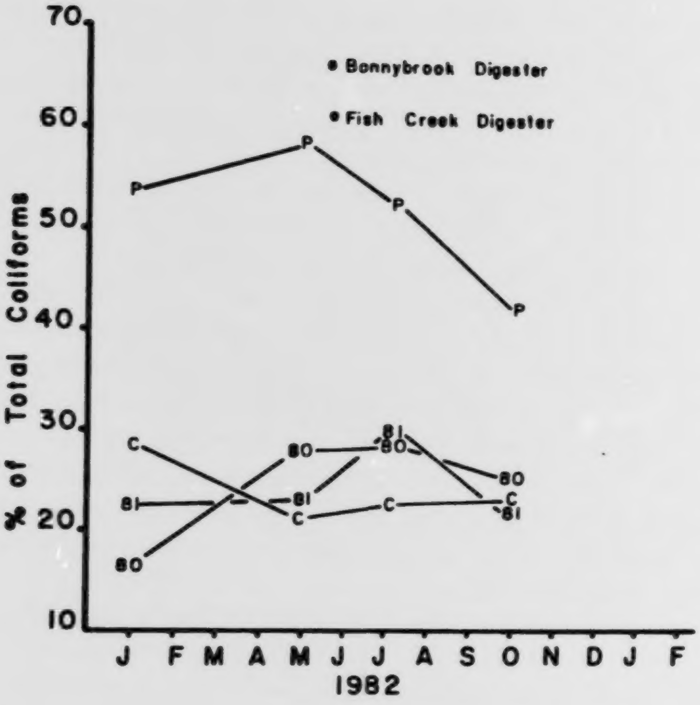


Figure 13. ECE percentages from the sludged fields and pasture.

Also noted in Figure 13, are the data for total coliforms isolated from the Calgary Bonnybrook and Fish Creek anaerobic mesophilic digesters. The significance of these data is that the percentage of total contamination of the pasture, though higher than the sludged and control fields, is somewhat lower than that found in the anaerobic digesters.

The organisms determined by colony identification from human faeces are similar to those from raw sludge, mesophilically digested sludge, and lagoon sludge (see section on bacterial analyses of sludges below). The situation, however, modifies after sludge has been placed on land (Table 19). When colonies identified are considered, it is evident that *E. coli* have not been found from 1980 or 1981 sludged fields, but they have been found from the control field and the pasture. It is likewise obvious that the nature of the total coliforms had modified radically, with *Enterobacter* and *Citrobacter* being predominant among the enteric forms. This modification of coliforms over time/environment has also been found by Kott (1977) during the examination of wastewater, and his results are reported in Table 20. Between 0-time and 56 days, the total coliform count fell from 10^7 to 10^3 organisms. This is consistent with previous work, but there was a significant modification of the relative percentages of total coliforms. *E. aerogenes*, not found at 0-time, was present later, implying a low initial concentration which subsequently increased.

It has been noted that there are distinct differences in percentages of total coliforms between the 1980/1981 control fields and the pasture (Figure 13). When the actual numbers of total coliforms are considered, a similar, but not identical, disparity results (Table 21) which again distinguished the fields from the pasture. When the actual numbers of total coliforms from the fields/pasture are compared with the numbers from the anaerobic digesters, an interesting picture emerges. During the seasons when the numbers of total coliforms are low from the fields/pasture (winter, summer and fall) the numbers of organisms are less than or nearly equal to those reported from the digesters (Fish Creek: 843 and 1111/g dry wt.; Bonnybrook: 314 and 320/g dry wt.). During the spring, the numbers of total coliforms from the fields/pasture increase dramatically (Table 21) and exceed by one or two orders of magnitude those reported (above) from the digesters.

Table19. Genera of bacteria isolated by season from sludged fields, control field and pasture.

	Winter				Spring			
	1980	1981	Pasture	Control	1980	1981	Pasture	Control
Escherichia	0	0	0	0	0	0	6	0
Citrobacter	26.1	21.3	9.4	0	18.9	20	36.4	35.5
Enterobacter/ *Klebsiella*	8.7	21.3	68.7	31.6	24.3	22.5	45.5	0
Hafnia	21.7	12.8	12.5	26.3	29.7	20	12.1	32.2
Serratia	43.5	44.7	9.4	42.1	27.1	37.5	0	32.2
Proteus/Providencia	0	0	0	0	0	0	0	0
Total Colonies	23	47	32	19	37	40	33	31
No Growth, Not Id.	3	3	3	31	3	0	7	9
ECE	16.1	23.1	53.5	27.8	26.5	22.5	57.8	20.4

continued...

Table 19. Continued.

		Summer				Fall		
	1980	1981	Pasture	Control	1980	1981	Pasture	Control
Escherichia	0	0	13.1	2.5	0	0	17.5	0
Citrobacter	38.5	12.8	26.3	5.0	22.5	8.1	22.5	38.5
Enterobacter/ Klebsiella	43.6	59	36.8	60.0	45	21.6	32.5	53.8
Hafnia	0	7.7	5.2	20.0	7.5	48.6	5	0
Serratia	17.9	20.5	13.1	0	17.5	21.6	12.5	7.7
Proteus/Providencia	0	0	5.2	0	0	0	10	0
Total Colonies	39	39	38	40	40	37	40	39
No Growth, Not Id.	1	1	2	0	0	3	0	1
ECE	25.6	28.5	52.5	22.5	25	21.6	42.5	23.1

continued...

Table 19. Concluded.

			Composite	
	1980	1981	Pasture	Control
Escherichia	0	0	9.2	0.63
Citrobacter	26.5	15.6	21.3	19.8
Enterobacter/ Klebsiella	30.4	31.1	45.9	36.5
Hafnia	14.7	22.3	8.7	19.6
Serratia	26.5	31.1	8.8	23.6
Proteus/Providencia	0	0	3.8	0
Total Colonies	139	163	143	129
ECE	23.3	23.9	51.6	23.4

Table 20. Percentage of coliforms of total isolates made by Kott (1977) from wastewaters.

	E. coli	*Ent. aerogenes*	other coliforms
0-time	83	0	6
21 days	37	12	45
56 days	8	29	58

Table 19. Concluded.

			Composite	
	1980	1981	Pasture	Control
Escherichia	0	0	9.2	0.63
Citrobacter	26.5	15.6	21.3	19.8
Enterobacter/ Klebsiella	30.4	31.1	45.9	36.5
Hafnia	14.7	22.3	8.7	19.6
Serratia	26.5	31.1	8.8	23.6
Proteus/Providencia	0	0	3.8	0
Total Colonies	139	163	143	129
ECE	23.3	23.9	51.6	23.4

Table 20. Percentage of coliforms of total isolates made by Kott (1977) from wastewaters.

	E. coli	*Ent. aerogenes*	other coliforms
0-time	83	0	6
21 days	37	12	45
56 days	8	29	58

Table 21. Numbers of total coliforms (ECE[a]) per g dry weight × 10^4.

	1980 Field	1981 Field	Pasture	Control Field
Winter	35	168	18	16
Spring	96 860	46 409	125 724	20 290
Summer	41	84	204	33
Fall	92	86	246	70
Composite	97 028	57 036	126 192	20 415

[a] ECE = *Escherichia coli*, *Citrobacter freundii*, and *Enterobacter aerogenes*

It seems evident that during the spring months total coliforms are capable of reproduction in sludged soils, control soil, and in pasture soil. As to the genera capable of this activity, the data in Table 22 should be compared. On the basis of these data, it is evident that *E. coli* probably neither survives for any extended period of time nor is capable of reproduction on sludged soil or the pasture. The presence of this species was only detected from the pasture and, to a much lesser extent, from the control field; its existence can be attributed to bovine or deer contamination.

C. freundii and *E. aerogenes* were certainly added to the sludged fields by the applied sludge but could also have been added from contamination by small animals and may have been present as normal inhabitants of the soil. The existence of these organisms from the control field and the pasture could be of multiple origin: as normal inhabitants of the soil, as contaminants applied by cattle and deer (a single *E. coli* was isolated from the control field in the summer), and from various small animals. Regardless of the source, two of the total coliforms, *C. freundii* and *E. aerogenes*, are capable of reproduction in soil when conditions are favourable.

6.2.3 The Secondary Pathogens

A number of organisms producing classically important diseases (primary pathogens, e.g., tuberculosis, salmonellosis), in man can be found in sewage waste disposal products. In addition, certain bacteria which have long been considered to be benign have recently been shown to be both pathogenic (Center for Disease Control 1978) and capable of existing in sludge. These bacteria may be referred to as secondary pathogens (those which attack immunologically weak hosts). Guentzel (1980) reports that the following genera of the Family Enterobacteriaceae fall into this category, *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter*, and reported finding them from effluent. A study by the Center for Disease Control (1978) confirmed the above genera as being of significance (except for *Citrobacter*) and extended the list by the addition of *Proteus*, *Providencia*, *Pseudomonas aeruginosa*, *Staphylococcus*, and group D *Streptococcus* (faecal streptococci).

Table 22. Comparison of ECE from digesters, digested sludge, and sludged fields.

	Percentages of total coliforms from digesters	Percentages of total coliforms from injected sludge	Combined percentages of total coliforms from sludged fields, control field and pasture
E. coli	71.1%	70%	5.4%
C. freundii	8.9%	10%	43.2%
E. aerogenes	20.0%	20%	51.4%

Leclerc (1977) and Dufour (1977) have indicated that *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter* are the most common inhabitants of the human gut amongst the Enterobacteriaceae. Dudley et al. (1980), Harding et al. (1981) and Guentzel (1977) have recorded these organisms as being the most common from sludge and effluent.

Dufour (1977) has analysed the microbial constituency of total coliform tests and found that *E. coli* represents about one-third of the isolates, *Klebsiella* 27%, and *Citrobacter*/*Enterobacter* 40%. Dufour (1977) likewise examined in excess of 1600 colonies from 78 domestic animals and found that, of the total coliforms, 94% were *E. coli* while the remainder were *Klebsiella*, *Citrobacter*/*Enterobacter*.

The genera of secondary pathogens isolated belonging to the Enterobacteriaceae are listed in Table 19 according to field and time of isolation. The relative percentages of genera, both when compared between the individual fields and seasonally, do not appear to demonstrate any particular pattern, but when *Escherichia*, *Citrobacter freundii* and *Enterobacter aerogenes* are enumerated and compared (Figure 13 and section dealing with the total coliforms), discreet differences between the sludged fields-control field and the pasture appear. Several interesting features do appear. All of the genera represented are considered to be secondary pathogens except *Hafnia*. *Proteus*/*Providencia* were rarely encountered. *Escherichia coli* was isolated (aside from MPN faecal coliform methods) only from the pasture and the control field but not from the sludged fields and no mention is made of the isolation of *Klebsiella*. *Enterobacter*-*Klebsiella* is used to enumerate the group. We were unable to distinguish environmental *Klebsiella* from *Enterobacter* by using the available diagnostic data (i.e., motility and ornithine decarboxylase reactions). Additionally, in the 8th edition of Bergey's Manual (Buchanan and Gibbons 1974), it is mentioned that strains of *Aerobacter* (*Enterobacter*) *aerogenes* capable of growth at 37°C were indistinguishable from *Klebsiella*.

It would also be well to consider the comments of others intimately acquainted with the identification of the members of the Enterobacteriaceae. For instance, Buchanan and Gibbons (1974, Bergey's Manual of Determinative Bacteriology) write regarding the genera of the Enterobacteriaceae, "There is increased evidence of sharing of characters ...and apparent intra- and inter-generic transformation, so that taxonomic opinion is moving toward a reduction in the number of genera" and, "There have been various suggestions for the combination of one or more of the genera Klebsiella, Enterobacter, Hafnia and Serratia." Pelczar et al. (1977) state "In a group such as this (Enterobacteriaceae), it is not surprising that there is an interrelationship among types of these bacteria and that many intermediate strains exist." Finally, Kott (1977) states regarding the coliforms "there are difficulties in isolation, identification and interpretation of the results." In summary, there are great difficulties in the isolation and identification of the members of the Enterobacteriaceae with which we are concerned and it might be suggested that computer analysis be employed. This suggestion is responded to by Farmer and Brenner (1977): "Even with a large battery of biochemical tests and computer analyses we find it difficult to assign some of the environmental strains to an existing species."

6.2.4 The Numbers of the Enterobacteriaceae: MacConkey Plate Counts

Table 23 shows the numbers of bacteria capable of growth on MacConkey agar which were found from the sludged fields, the pasture, and the control field during the seasonal samplings in 1982. In addition to the total counts, the percentages of oxidase negative colonies are recorded and these are presumed to belong to the Enterobacteriaceae; the oxidase positive remainder could probably be allocated to several bacterial genera. Because representative colonies were found to be Gram negative, it is presumed that the organisms are potentially allergenic because of their component lipopolysaccharides (LPS).

Obviously, LPS-containing organisms are present from all fields. A significant feature, however, is the cyclic nature of the population regardless of field from which recovered. During the winter, the numbers are small but during the following spring there is a population explosion with an equally dramatic population reduction during the summer and fall months.

The reasons for these seasonal modifications of numbers have not been ascertained but it can be presumed that interspecific competition between the Enterobacteriaceae and other

Table 23. The sludged fields: MacConkey agar counts (numbers/g dry wt x 10^4) and percentages of oxidase negative colonies.

	1980 Field		1981 Field		Pasture		Control Field	
	MacConkey Numbers	Oxidase Neg. %	MacConkey Numbers	Oxidase Neg. %	MacConkey Numbers	Oxidase Neg. %	MacConkey Numbers	Oxidase Neg. %
Winter	291	78.6	38.2	61.5	37.2	78.9	63.7	92.8
	250	81.2	2 180	69.4	51	75	70.1	84.1
Average	271	79.9	1 109	65.5	44.1	76.9	66.9	88.5
Spring	752 940	89.6	363 636	89.7	220 285	79	97 421	95.1
	83 432	85.2	95 238	90.1	314 810	83.5	177 710	49.4
Average	418 186	87.4	229 437	89.9	267 547	81.3	137 565	72.3
Summer	171	81	387	71.5	471	86	202	81.8
	255	70.6	381	82.1	426	87.1	168	76.6
Average	213	75.8	384	76.8	449	86.6	185	79.2
Fall	303	81.1	116	87.5	207	83.5	450	85.5
	588	84.3	840	78.6	1 181	83.1	260	85.0
Average	446	82.7	478	83.1	694	83.3	355	85.3

soil forms could be contributory. A more detailed discussion of such competition, and other factors, may be found in the bacterial literature review.

It would appear that, because of the similarity in numbers (less than one order of magnitude), the sludged fields and the pasture approach the background level of the control field with regard to Gram negative, and potentially allergenic, organisms.

6.2.5 The Primary Pathogens

Three primary pathogens were searched for during the tenure of this project. Analyses for *Salmonella* were made during all three years while emphasis on the isolation of *Staphylococcus* and *Mycobacterium* was restricted to the terminal year.

Although pathogens are found in effluent, it is the contention of Van der Drift et al. (1977) that bacteria tend to be differentially separated from the liquid phase to sludge by adsorption to the solid biomass. Even though various bacteria (including pathogens) can concentrate in sludge by physical means, their numbers are reduced (pathogens) by 99.8% during anaerobic digestion (Gritzuk 1973). The numbers of pathogens capable of remaining viable on sludged land, however, appears to be negligible as indicated by the following data, and such low viability is substantiated by various authors and noted in the literature review.

6.2.5.1 *Salmonella*. These organisms were never found from sludged fields, the pasture, or the control field. They were recovered elsewhere: raw sewage (Bonnybrook and Crossfield), raw sludge (Fish Creek), and mesophilic digesters (Fish Creek and Bonnybrook). It seems evident, then, that *Salmonella* pose no potential health hazards on sludged land, at least with reference to the analytical methods employed by the Kananaskis group.

6.2.5.2 *Staphylococcus*. No Gram positive cocci were isolated. Inoculated plates of Mannitol Salt Agar, however, did show the presence of varying numbers of yellow, mannitol fermenting organisms capable of growth at 35 to 37°C, but microscopic examinations showed that all were large, asporulate Gram positive rods. It would seem then, that if any reasonable and accurate analysis of Staphylococcus is to be conducted from sludged lands, a new, and more restrictive type of medium will have to be formulated which can prevent the growth of these contaminating soil organisms. Evans (1976) refers to a paper presented at an EPA workshop by Olivieri and Riggio in which similar results were found (from stormwater) with a similar suggestion for a more restrictive type of isolation medium.

6.2.5.3 Mycobacterium. Acid fast organisms were isolated from time to time from sludged fields (Table 24) but because all cultures were contaminated with other micro-organisms we were unable to obtain pure cultures for further testing. Our methods of treatment of sample so as to remove contaminants was the classical NaOH incubation as specified by various authors (e.g., Collins and Lyne 1976). If we had used the benzalkonium suppressive treatment as advocated by Guentzel (1977) and Dudley et al. (1980), it is conceivable that our results might have been more rewarding. Larkin et al. (1977) however mention that M. tuberculosis in sewage is not easily estimated because of the difficulty of separating the organisms from other bacteria. The same authors were unable to detect M. tuberculosis from seeded sludged soil after a period of 29 days because of the masking effect of other soil organisms.

6.2.6 Fluorescent Pseudomonas

These organisms were isolated from the 1982 plot seven days after sludging but not subsequently. Samples from the 1980 and 1981 sludged field and the pasture were negative until the fall of 1982 when both became positive. Pseudomonas was not found from the control field or the pasture during the analytical period.

The Center for Disease Control (1978) has found that Pseudomonas aeruginosa is responsible for some 7.4% of all nosocomial infections, and Colwell et al. (1978) believe that Pseudomonas presents a potential health hazard. Kenner et al. (1971) notes the organism in sewage products finding 34 P. aeruginosa/100 mL of digested sludge while Rand et al. (1976) state that, in addition to being an important indicator species for determining contamination of recreational waters, Pseudomonas is common in sewage.

soil forms could be contributory. A more detailed discussion of such competition, and other factors, may be found in the bacterial literature review.

It would appear that, because of the similarity in numbers (less than one order of magnitude), the sludged fields and the pasture approach the background level of the control field with regard to Gram negative, and potentially allergenic, organisms.

6.2.5 The Primary Pathogens

Three primary pathogens were searched for during the tenure of this project. Analyses for *Salmonella* were made during all three years while emphasis on the isolation of *Staphylococcus* and *Mycobacterium* was restricted to the terminal year.

Although pathogens are found in effluent, it is the contention of Van der Drift et al. (1977) that bacteria tend to be differentially separated from the liquid phase to sludge by adsorption to the solid biomass. Even though various bacteria (including pathogens) can concentrate in sludge by physical means, their numbers are reduced (pathogens) by 99.8% during anaerobic digestion (Gritzuk 1973). The numbers of pathogens capable of remaining viable on sludged land, however, appears to be negligible as indicated by the following data, and such low viability is substantiated by various authors and noted in the literature review.

6.2.5.1 *Salmonella*. These organisms were never found from sludged fields, the pasture, or the control field. They were recovered elsewhere: raw sewage (Bonnybrook and Crossfield), raw sludge (Fish Creek), and mesophilic digesters (Fish Creek and Bonnybrook). It seems evident, then, that *Salmonella* pose no potential health hazards on sludged land, at least with reference to the analytical methods employed by the Kananaskis group.

6.2.5.2 *Staphylococcus*. No Gram positive cocci were isolated. Inoculated plates of Mannitol Salt Agar, however, did show the presence of varying numbers of yellow, mannitol fermenting organisms capable of growth at 35 to 37°C, but microscopic examinations showed that all were large, asporulate Gram positive rods. It would seem then, that if any reasonable and accurate analysis of *Staphylococcus* is to be conducted from sludged lands, a new, and more restrictive type of medium will have to be formulated which can prevent the growth of these contaminating soil organisms. Evans (1976) refers to a paper presented at an EPA workshop by Olivieri and Riggio in which similar results were found (from stormwater) with a similar suggestion for a more restrictive type of isolation medium.

6.2.5.3 *Mycobacterium*. Acid fast organisms were isolated from time to time from sludged fields (Table 24) but because all cultures were contaminated with other micro-organisms we were unable to obtain pure cultures for further testing. Our methods of treatment of sample so as to remove contaminants was the classical NaOH incubation as specified by various authors (e.g., Collins and Lyne 1976). If we had used the benzalkonium suppressive treatment as advocated by Guentzel (1977) and Dudley et al. (1980), it is conceivable that our results might have been more rewarding. Larkin et al. (1977) however mention that *M. tuberculosis* in sewage is not easily estimated because of the difficulty of separating the organisms from other bacteria. The same authors were unable to detect *M. tuberculosis* from seeded sludged soil after a period of 29 days because of the masking effect of other soil organisms.

6.2.6 Fluorescent *Pseudomonas*

These organisms were isolated from the 1982 plot seven days after sludging but not subsequently. Samples from the 1980 and 1981 sludged field and the pasture were negative until the fall of 1982 when both became positive. *Pseudomonas* was not found from the control field or the pasture during the analytical period.

The Center for Disease Control (1978) has found that *Pseudomonas aeruginosa* is responsible for some 7.4% of *all* nosocomial infections, and Colwell et al. (1978) believe that *Pseudomonas* presents a potential health hazard. Kenner et al. (1971) notes the organism in sewage products finding 34 *P. aeruginosa*/100 mL of digested sludge while Rand et al. (1976) state that, in addition to being an important indicator species for determining contamination of recreational waters, *Pseudomonas* is common in sewage.

Table 24. Isolation of acid fast bacteria from the fields.

	1980 Field	1981 Field	Pasture	Control Field	1982 Field
Winter	0	0	+	0	N.D.[a]
Spring	+	0	+	0	N.D.
Summer	0	+	+	0	+
Fall	N.D.	N.D.	N.D.	N.D.	0

[a] N.D. = Not Determined.

Buchanan and Gibbons (Bergey's Manual of Determinative Bacteriology) state that _P. aeruginosa_ can be isolated from soil and water. This is in agreement with Hugh and Gilardi (1974) and McFeters et al. (1978) who report that _Pseudomonas_ persists in water for long periods of time. To the contrary, Kenner et al. (1971) state that there are no _P. aeruginosa_ in unsludged soil and that the organism can exist in the top 2 inches of land applied sludge for 21 weeks. Bonde (1963) confirms the premise, believing that the presence of the organism implies faecal contamination.

Regarding the presence of _P. aeruginosa_ in intestinal contents, Sutter and Hurst (1967) found about 12% of the faeces of health adults were contaminated while Hugh and Gilardi (1974) indicated that the organism is not a common inhabitant of the human gut, with a 10% rate being encountered. Wheater et al. (1980) recorded a 15% rate of infection from humans and reconfirmed the findings of Smith (1961) by noting that non-human mammals (except cats and dogs) displayed an appreciable lesser degree of habitation.

6.3 THE 1982 SLUDGED FIELD

Even though, as explained earlier, it is improbable that surface sludged but undisced land as found on the 1982 plot would remain undisced and fallow, a bacteriological study might provide valuable information as to the fate of the organisms during the first six to seven months after sludging. The reason that this information could be applicable is that the scheme for microbial analyses was not fully functional until the winter of 1981-82. Possibly, then, the events noted during the early history of the 1982 field could be applied to the early history of the 1981 field.

The data for the 1982 surface sludged plot (over grass) are noted in Table 25. It is obvious that there is a decrease in excess of two orders of magnitude in the numbers of MacConkey positive organisms and total coliforms between 0-time plus seven days and 44 days after sludge application. These low numbers corresponded to that time when moisture content of the sludge was at the lowest recorded percentage (8.2%). Following this low, subsequent samplings revealed higher moisture contents (17 to 31%) with an immediate increase in numbers of both total coliforms and MacConkey positive organisms, the peak occurring 84 days after

Table 25. 1982 Surface sludged plot.

	Lagoon Sludge	Plot Sludged June 22	7 Days June 29	43 Days Aug. 4	84 Days Sept. 14	118 Days Oct. 18	127 Days Oct. 27	147 Days Nov. 16	154 Days Nov. 23	217 Days Jan. 24	224 Days Jan. 31
Escherichia	35.3%		60	63.2	10	72.2	16.7	15	15.8	0	0
Citrobacter	29.4%		5	0	5	11.1	5.5	20	26.3	5	15
Enterobacter/ Klebsiella	5.9%		0	21	45	16.7	77.7	55	36.8	35	40
Hafnia	0		0	0	30	0	0	0	0	20	10
Serratia	0		0	15.8	10	0	0	10	21.1	40	35
Proteus/ Providencia	29.4%		35	0	0	0	0	0	0	0	0
No. of Colonies	20		20	20	20	18	18	20	19	20	20
MacConkey Count #/g dry wt x 10^4	TNTC[a]		415 000	1 743	30 400	24 700	24 600	17 400	8100	3185	2690
% Oxidase Negative	N.D.[b]		69.4	84	81.1	87.7	86.6	93.5	87.3	85.9	88.6
% ECE	58.8		65	78.9	40	72.2	66.7	35	42	30	30
# ECE x 10^4	N.D.		187 000	1155	9860	15 600	14 200	5700	2970	955	715

[a] TNTC = Too Numerous to Count.

[b] N.D. = Not Determined.

sludging. Subsequently, the numbers of MacConkey positive organisms declined consistently, achieving a low of 2690 X ^{4}/g dry wt. on January 31, 1983. Both the percentages and the numbers of total coliforms (ECE) declined over time (with an occasional remission along the way) to a low point, again on January 31 (244 days after sludging).

Escherichia coli were found consistently up to November 23 (154 days after sludging) but later (217 and 224 days post sludging) they were not isolated. Faecal coliforms were frequently, but not consistently, detected up to 154 days but not later. *Staphylococcus* and *Salmonella* were not found at any time while fluorescent *Pseudomonas* were recovered twice: 7 and 118 days post sludging. In general, and as previously noted, the prime features responsible for the decrease of numbers and the modification of species percentages of constituent sludge organisms are moisture, temperature, and competition from the microflora and fauna of the soil. Because the sludge was applied on grass, it is unlikely that there was more than minimal interaction with the normal biotic community of the soil except in those instances of soil-sludge interface; in such circumstances, little mixing occurred, only physical opposition.

When sludge is applied to grass and reasonable moisture content and temperature are maintained, it appears likely that the mesophilic organisms normally resident in sludge will inter-compete and that the relative proportions of species will remain stable such as was noted in raw, digested, and lagoon sludges (see Table 26 for similarities). *Proteus/Providencia*, however, disappear. When temperatures fall to less than mesophilic levels (but still above freezing), the organisms are no longer able to successfully inter-compete with the psychrophilic forms which then assume ascendancy, and their activities result in the reduction of numbers and variations of types of Enterobacteriaceae; in addition, it may be presumed that such circumstances can result in the formation of stressed cells. When freezing of the sludge occurs, metabolic activity is minimal and the numbers and the types of mesophilic organisms (specifically the Enterobacteriaceae) decrease and/or modify. This is presumably the result of freezing upon cell wall integrity of the previously stressed cells.

Table 26. Genera of bacteria from sludge.

	Percent of Total Colonies Identified						
			Fish Creek		*Bonnybrook*		
	Raw Sludge #1	Raw Sludge #2	Dig. Sludge #1	Dig. Sludge #2	Dig. Sludge #1	Dig. Sludge #2	Lagoon Sludge
Escherichia	35.3	58.8	31.6	42.1	56.3	47.4	35.3
Citrobacter	17.6	29.4	15.8	5.2	18.8	10.5	29.4
Enterobacter/ *Klebsiella*	23.5	11.7	15.8	26.3	18.8	10.5	5.9
Hafnia	0	0	0	0	0	0	0
Serratia	0	0	0	0	6.3	10.5	0
Proteus/ *Providencia*	23.6	0	36.8	21.0	0	21.0	29.4
Total Colonies	17	17	19	19	16	19	17
No Growth, Not Id.	3	3	1	1	4	1	3
E.C.E.	88.9	58.8	47.4	68.4	63.2	68.8	58.8

It is probable that the history of the bacteria from the sludged plot does not simulate that which would have occurred in the pre-winter months of the 1981 field. Presumably, because of competition with normal soil mesophiles, the numbers and types of the Enterobacteriaceae would have reached winter levels at an earlier date. Even though the early history of the 1982 plot and the 1981 fields are presumably dissimilar, the end products as noted in the January samplings are quite similar (see Tables 19 and 25).

6.4 BACTERIAL ANALYSES OF RAW, DIGESTED, AND LAGOON SLUDGE FROM THE BONNYBROOK AND FISH CREEK PLANTS

The above noted analytical categories are included under a single heading as systematic study can indicate microbial modifications (numbers) occurring in sludge from the incipient, raw state, through treatment and, ultimately, to storage. These data, particularly from lagoon sludge, can also be applied and compared with the events occurring on sludged fields following application. In addition, a more realistic appraisal of potential health hazards of the pasture, and apparent lack of health hazard of the control field, can be ascertained by comparing the microbial constituencies.

Two samples each of raw and digester sludge were examined from the Fish Creek plant, the latter 21 days after the raw material had been placed in the digester. Raw sludge was not examined from the Bonnybrook plant but two samples of digester sludge were investigated. One sample of lagoon sludge was processed.

6.4.1 Faecal Coliforms

Faecal coliforms were isolated by the MPN method from all samples.

6.4.2 Total Coliforms

Table 26 indicates, under the title ECE (Escherichia-Citrobacter freundii-Enterobacter aerogenes), the percentage of organisms identified which have been designated as total coliforms. These organisms averaged 64.9% (47.4 to 88.9%) of the colonies identified and, with the exception of the lower figure, exceed the numbers recorded from the pasture (Figure 13). The figures are twice or more as great as those found from the 1980 and 1981 sludged fields and from the control field (Figure 13).

6.4.3 The Secondary Pathogens

Table 26 shows the species, some of which may be considered to be secondary pathogens, isolated and should be compared with the forms found from the sludged and control fields and the pasture (Table 19). It is obvious that the combined percentages of *Escherichia* and *Proteus/Providencia* predominate, a reversal of the situation from the fields.

6.4.4 The Numbers of the Enterobacteriaceae: MacConkey Plate Counts

The data appearing below show the number of presumed Enterobacteriaceae and total coliforms found for each sample was determined by growth on MacConkey agar and the results of the oxidase test. It is obvious that the mesophilic, anaerobic digester significantly reduces the numbers of such organisms (by 89.8 to 99.10%). It is equally obvious that, following storage in the lagoon, there is an increase of numbers with *Escherichia* and *Proteus/Providencia* still predominating (Table 27).

6.4.5 The Primary Pathogens

Coagulase positive *Staphylococcus* were found from both samples of raw sludge; they were not found from digester sludge nor from lagoon sludge. *Salmonella* spp. was isolated from Bonnybrook digester sludge, and Fish Creek raw and digester sludge. *Mycobacterium* was not found.

6.4.6 Fluorescent Pseudomonas

Fluorescent pseudomonads (37°) were found from all samples.

6.5 BURIED/INJECTED SLUDGE

Because sludge is being injected, rather than spread, on some fields in the Shepard area, it seemed essential to conduct studies on the sub-surface fate of the organisms found in the product.

Table 27. Microbiological data from Bonnybrook and Fish Creek sludge.

	Raw	Raw	Fish Creek Digester	Fish Creek Digester	Bonnybrook Digester	Bonnybrook Digester	Lagoon
MacConkey Count $\times 10^4$	34 500	228 000	3550	2070	627	609	TNTC[a] 10
Oxidase Negative percent	90	92.1	69	78.5	79.4	78.9	N.D.[b]
Enterobacteriaceae Count $\times 10^4$	31 050	210 000	2450	1625	498	480	
% Reduction			89.8	99.1			
Total Coliform Count $\times 10^4$	27 603	123 473	834	1111	314	330	

[a] TNTC = Too Numerous to Count.

[b] N.D. = Not Determined.

Two studies were done. In one, lagoon sludge was *hand-buried* 20 cm below the surface on the unsludged and untouched periphery of the 1981 field during September 1981. Secondly, *machine-injected* (15 cm) sludge was investigated seven days after placement in June 1982. Extensive microbiological examinations were conducted at this latter time and, because these methods were not as yet perfected for analysis of the *hand-buried* sludge, it was decided that the 0-time + 7 day results found for the *injected sludge* would be similar to that of the hand-buried sludge for the purposes of comparisons over time.

The *hand-buried sludge* was untouched until sampling. Unfortunately, but one sample was secured (258 days after burying); other attempts at 277 and 313 days failed to locate the seam.

The *injected sludge* field was cultivated during the process of application. It was then disced and allowed to lie fallow throughout the summer when it was again disced and planted with rye. Instead of attempting to locate any intact sludge, (aside from the initial analysis 7 days after injection) most of which had been fragmented and re-distributed during discing, surface samples were taken at the following times:

1. 19 August - 66 days after injection, and
   55 days after first discing
2. 13 September - 91 days after injection, and
   16 days after second discing
3. 19 October - 127 days after injection, and
   52 days after second discing
4. 26 October - 123 days after injection, and
   59 days after second discing
5. 15 November - 143 days after injection, and
   70 days after second discing
6. 22 November - 150 days after injection, and
   77 days after second discing

Table 28 shows a sequential but theoretical series of events dealing with the microbial history of *hand-buried sludge*. Because, as previously mentioned, the bacteriological methods had not been perfected at the time of burying, a later analysis of lagoon sludge has been inserted to represent 0-time (or the sludge

Table 28. Hand-buried sludge.

	0-Time Lagoon Sludge	7 Days 1982 Injected Sludge	Buried After 258 Days
Escherichia	35.3	36.8	20
Citrobacter	29.4	42.1	35
Klebsiella/ *Enterobacter*	5.9	21	35
Hafnia	0	0	10
Serratia	0	0	0
Proteus/ *Providencia*	29.4	0	0
Total number Not id.	20	20	20
ECE	58.8	55	55
MacConkey count No./g dry weight	$>50\ 000\ 000 \times 10^4$	$260\ 000 \times 10^4$	2033×10^4
% oxidase negative	N.D.[a]	70.2	90.2
Numbers of ECE	N.D.	$100\ 400 \times 10^4$	1008×10^4
Pseudomonas	+	0	0
Staphylococcus	0	0	0
Mycobacterium	+	0	0
Salmonella	C	0	0
Faecal coliforms-MPN	+	+	+

[a] N.D. = Not Determined.

which was buried). The results recorded for injected sludge 7 days after placement can theoretically substitute for a similar time interval for the hand-buried sludge. The third column of Table 28 represents the actual bacteriological data for hand-buried sludge 258 days after deposition.

Five features are noteworthy and should be commented upon: (1) the numbers of the Enterobacteriaceae in buried sludge are dramatically reduced over time (2 orders of magnitude); (2) the *Proteus/Providencia* group disappears after burying; (3) fluorescent pseudomonads disappear; (4) the percentage of *Escherichia* isolates remains high in sludge buried for 258 days as does the presence of faecal coliforms; and (5) the percentage of total coliforms (ECE) is similar to that from lagoon sludge and identical with that of the 7 day injected sludge. The numbers of total coliforms (ECE), however, is two orders of magnitude less than found from 0-time + 7 days injected sludge, and in excess of two orders of magnitude less than the numbers recorded from the pasture during the spring samplings. The implication of these data is that,with regard to the presence of *Escherichia coli*, the buried sludge resembles lagoon sludge. The percentages of total coliforms of both lagoon and 258 day buried sludge are similar, but the latter is two orders of magnitude less than the former and, even though discing would contaminate the surface, the total numbers of organisms, including *E. coli*, would be relatively small and they would decrease over time as noted in the following section dealing with injected sludge.

The bacteriological data for *injected sludge* is presented in Table 29. Aside from the 0-time + 7 days information which represents substantially that which was buried, the data can be construed representing two separate but related events: (1) the fate of the organisms present in the injected sludge brought to the surface by the first discing and were exposed to summer conditions; and (2) the fate of the organisms in the residual injected sludge contaminating the surface as the result of the second discing and were exposed to late summer and fall weather conditions.

Fifty-five days after the first discing, the total numbers of bacteria capable of growth on MacConkey agar had been reduced by more than three orders of magnitude. The percentage of

Table 29. Injected sludge.

	Digesters	Lagoon	Injected June 14 7 Days June 21	First Discing June 25 66 Days Aug. 19	Second Discing Aug. 28 86 Days Sept. 13	121 Days Oct. 19	128 Days Oct. 26	146 Days Nov. 15	153 Days Nov. 22
Escherichia	43.8%	35.3	36.8	15	0	0	0	0	0
Citrobacter	12.3%	29.4	42.1	40	0	21.1	0	10	25
Enterobacter/ Klebsiella	19.2%	5.9	21.1	25	70	57.8	55	90	25
Hafnia	0	0	0	20	30	21.1	0	0	20
Serratia	4.1%	0	0	0	0	0	45	0	30
Proteus/ Providencia	20.1%	29.4	0	0	0	0	0	20	20
# of Colonies	20	29	19	20	20	19	20	20	20
MacConkey Count #/g dry wt x 10^4	17 890	TNTC[a]	260 000	50	140	138	80.1	9.5	46.1
% Oxidase negative	N.D.[b]	N.D.	70.2	71.9	79	77.1	95.2	88	70
% ECE	61.9	58.8	55	20	40	42.1	25	10	10
No. ECE x 10^4	N.D.	N.D.	100 400	7.2	44	45	19	0.8	3.2

[a] TNTC = Too Numerous to Count.

[b] N.D. = Not Determined.

total coliforms was similar to those of the 1980 and 1981 sludged fields while the numbers of total coliforms were similar but somewhat less than noted in the fields. *Escherichia coli*, however, were identified from isolated colonies but the numbers were small (5.4×10^4/dry wt. g) as compared to 67 000 $\times 10^4$/dry wt. g for the 0-time + 7 days injected sludge. It may be presumed that, had not a second discing occurred, the numbers of *E. coli* would have further diminished over time. *Proteus/Providencia* were not identified nor were they found from the injected sludge.

The second discing recontaminated the surface soil with MacConkey positive bacteria but of a magnitude (3 to 4 orders) less than the numbers found from the injected sludge. Neither *E. coli* nor *Proteus/Providencia* were isolated. The percentages of total coliforms 16 and 52 days following discing exceeded those noted from the surface sludged fields but subsequent examinations (59, 70 and 77 days) revealed that the percentages of total coliforms had fallen to field levels. The numbers of total coliforms likewise decreased to proportionally small numbers and also resembled the sludged fields and the control field.

Probably the still-buried sludge following the first discing was not in the intact mass as it was upon injection. The proportions of the constituent microbial species were then modified by the resident flora and various physiochemical factors as the bulk of the protective sludge mass had been disturbed. The microbial modification of the sludge organisms resulted in the loss of *Escherichia* and the presumed overgrowth of *Enterobacter* which contributed to the high ECE; this loss of *Escherichia* and overgrowth of *Enterobacter* has been reported by Kott (1977). *Salmonella*, *Staphylococcus*, and *Mycobacterium* spp. were not found. *Pseudomonas* was recovered but once (127 days after injection and 52 days after the second discing), while faecal coliforms were noted from the sludge on but one occasion, 7 days after deposition.

6.6 THE VIRUSES

Because the facilities necessary to conduct viral analyses were lacking at the Kananaskis Centre, it was necessary to make other arrangements to perform this task. Through the co-operation of Dr. L. Sekla, Cadham Provincial Laboratories, Winnipeg, various samples were examined for the presence of viruses. All samples were negative. The areas examined were:

1. 15 samples of the 1981 field 5 months after sludging;
2. 15 samples of the 1982 plot 7 days after sludging;
3. 5 samples of the lagoon sludge; age unknown;
4. 3 samples of mesophilic digester sludge;
5. 3 samples of the 1980 field 18 months after sludging;
6. 7 pasture samples;
7. 8 control field samples.

A virus literature review and the methods used for viral analysis are included in the second annual report (Lehmann et al. 1982). In addition, the subject of the occurrence and detection of viruses on sludged land has been exhaustively reviewed by Sattar (in press) and his conclusions are summarized below.

Over 100 enteric viruses have been identified to date and many more probably will be identified in the coming years. There are, however, many technical problems presented by the virological examination of sludge. The first is the difficulty of eluting the viruses from particles. Once they are eluted, the problem of contamination with bacteria and fungi remains. Sludge is particularly toxic to host cell cultures so that additional detoxification steps must be employed. Finally, there is no known host cell line with a sufficiently wide range of susceptability to be used for all enteric virus assays. Because of these difficulties, it is impossible to compare the results of various researchers without a good deal of interpretation and it is generally agreed that virus assay methods are inadequate. Further methodological research is badly needed in this field.

Even during adequate treatment of raw sewage, there is usually only a modest 1 to 2 $\log_{10}$ reduction in virus content. Viruses become particle associated in sludge and, later, in soil. Particle association generally enhances survival without affecting the potential for infectivity. The recovery and potential health hazard of viruses in soil therefore depends on the source of sewage, the characteristics of the soil to which viruses are applied, and the prevalence of viruses in the contributing population of man and animals. Viruses are known to bind easily to clays and it has been shown that the presence of water soluble humic material can inhibit the binding capacity of soils. Soils containing large amounts of humic organic matter are therefore not recommended for sludge disposal operations, at least from a virological point of view.

The survival of viruses on soil depends mostly on temperature and moisture conditions. Increases in temperature cause reduced viability because of moisture depletion and increases in metabolic activities of other attendant microbiota. Extremes of temperature can cause protein denaturation. Viruses trapped a few cm below the soil surface can survive for periods up to several months, at least when they are protected from sunlight and moisture and temperature extremes. Climatic conditions are therefore of great importance. It is also known that the standard coliform indicator tests bear little relation to the presence of viruses. Nevertheless, virus survival on land appears to compare, over time, most closely with that of bacteria (such as the salmonellae) rather than that of the parasites and their eggs.

Perhaps the most pressing problem which remains unanswered is the degree to which viruses contaminate groundwater (in which they may survive for long periods). Viruses tend to become concentrated in the upper soil horizons because of particle association, but cases of viral movement through groundwater resulting in the spread of disease have been documented. To the contrary, some studies have been unable to demonstrate the movement of viruses through soil. The evidence is contradictory and emphasizes the need for further research into the mechanisms of particle association in soils. Because viruses tend to accumulate in the upper soil horizons, food crops may become contaminated. Periods of time up to three years after sludging have been recommended (by the U.S. Food and Drug Administration) before sludge amended soil is used for the cultivation of crops to be eaten raw. It has also been suggested that even crops to be cooked should observe a similar waiting period because of the danger of cross-contamination of foods during processing. Viruses have been shown to be capable of surviving on plant material longer than the time required for processing and distribution to the consumer. Animals grazing on sludge amended pasture land are also at risk and may spread viral disease to human consumers or even to wild animals. This possibility must also be considered with respect to bacteriological pathogens but less so with helminths.

In conclusion, while there have been no documented cases of the spread of viral disease through the land application of treated sludge, cases have been reported from the irrigation of soil with raw sewage and from groundwater contamination. The inadequacy of reporting procedures and the lack of good epidemiological studies have confused this question. On the other hand, the mere presence of viruses on soil does not necessarily mean that a health hazard is present and it has been stated in the literature that the land disposal of sewage sludge is much safer than discharge to water bodies.

7. GENERAL DISCUSSION

Four areas of the investigation deserve comment: (1) the 1980 and 1981 sludged fields, control field and the pasture; (2) the 1982 sludged plot; (3) the digesters; and (4) buried/injected sludge. These topics will be discussed separately, but they tend to be inter-related, and an attempt will be made to combine the data into a cohesive package.

7.1 THE 1982 FIELD

Sludge applied to grass and allowed to remain in situ without further processing revealed that this method of application was efficient in the removal of parasite stages within less than two months. The bacteriology of this method, however, indicated that the procedure was inefficient with regard to the removal of Escherichia coli as these organisms were recovered throughout the summer and fall and disappeared only after several months of freezing temperatures. Their presence on grassland could constitute a potential health hazard (particularly to livestock), and an entire year of grazing would be lost if eradication of this organism was to be equated with minimal health hazard.

It is not known whether or not the microbial patterns noted for the first seven to nine months following sludge application simulated those that occurred in surface sludged and disced fields, or injected and disced fields. It is speculated, however, that the patterns differed because the grass applied material was not acted upon by indigenous soil microorganisms as was the disced sludge. Regardless of the mode of application and subsequent treatment, the microbial constituencies of all systems became similar after overwintering.

7.2 THE 1980 AND 1981 SLUDGED FIELDS, THE CONTROL FIELD AND THE PASTURE

The main purpose of this exercise was to determine if and when the surface sludged fields failed to present a potential health problem. This would occur when the sludged fields approached the background level displayed by the control field which, of necessity and by definition, must be considered to present no potential health hazard. Parenthetically, it should again be noted that the control field was exposed to contamination by wild animals, but that it had never been sludged nor had it been grazed for at least three years prior to examination.

The various facets of the study are described and discussed below.

7.2.1 The Parasites

The treatment afforded the 1981 sludged field (summer fallow following sludging and discing) rendered this plot parasite egg/larvae free in a matter of four months; in this respect, this field was superior to the control field. The 1980 sludged field was, after one year of cultivation, similar to the control field, and it displayed few parasite stages after an additional year.

The pasture presents a different picture in that it consistently shows a low-medium level of parasite eggs which are about twice as numerous as found from the control field. Presumably both fields are contaminated by deer (and other wild animals) which accounts for the level noted in the control field; the additional eggs from the pasture are presumably the result of contamination by cattle.

7.2.2 Faecal Coliforms (MPN)

The isolation of faecal coliforms is erratic and unpredictable. The literature tends to be controversial regarding the longevity of these organisms in soil. Because of this dispute over longevity and ability to grow in soil, the potential health hazard must remain mute.

7.2.3 The Total (Indicator) Coliforms

Inasmuch as these organisms (ECE = *Escherichia coli*, *Citrobacter freundii*, *Enterobacter aerogenes*) are delineated as being of significance because of their relationship (greater or lesser) to faecal contamination, the percentage of the population and the numbers of such organisms are of great importance.

Using the percentage of total coliforms as a measure, it is evident that the 1980 and 1981 sludged fields are returned to background level over 20 months and 9 months respectively. We do not know the microbial history of the 1980 field for the first 20 months, or the 1981 field for the first nine months as our microbial methods were not applied during these intervals.

There is a distinct difference between the sludged/control fields and the pasture. In the latter, the percentages of ECE isolates is approximately doubled and the numbers approach those encountered from the anaerobic digesters.

The actual numbers of total coliforms shows disparity between the fields (1980, 1981, and control) and the pasture, with the latter displaying about twice the number. Additionally, during the winter, summer, and fall, the numbers of total coliforms are less than that recorded from the anaerobic digesters, but in the spring the numbers from all fields and the pasture exceed (by >1 to >2 orders of magnitude) the numbers recorded from the digesters. The predominant organisms were *Citrobacter freundii* and *Enterobacter aerogenes*; *Escherichia coli* was found only from the control field (rarely) and the pasture (more commonly), and presumably its presence is the result of on-going contamination by resident wild and domestic animals. The implication, then, is that *C. freundii* and *E. aerogenes* are capable of growth on land regardless of their origin while *E. coli* probably does not survive or, at least, does not multiply on land.

7.2.4 Secondary Pathogens

Of the members of the Enterobacteriaceae noted by the Center for Disease Control (1978) as being secondary pathogens and producing nosocomial infections, 66.3% are attributed to *E. coli* (47.3%) and to *Proteus/Providencia* (19%). None of these organisms were isolated during the 1982 investigation from either the 1980 nor the 1981 sludged fields. *Escherichia coli* was found from both the control field and the pasture while *Proteus/Providencia* were recovered, in addition, from the pasture.

Klebsiella (accounting for 18.5% of Enterobacteriaceae nosocomial infections) were not identified from any source. It is conceivable that either analytical error was responsible for this situation, or that the organisms were not present. The keys employed to distinguish *Klebsiella* were lack of motility and decarboxylation of ornithine, and these diagnostic features never occurred simultaneously. Should strains of *Klebsiella* have been isolated and not identified as such, they would have been relegated to *Enterobacter*.

Enterobacter and Serratia (yielding a combined 15.1% of recorded infections) constituted an average of 58.5% of all genera of organisms identified during this study. Hafnia (16.3% of the total) is not identified as a secondary pathogen by CDC nor is Citrobacter (20.8% of the total) though the latter is so noted by Guentzel (1980).

The evidence indicates, then, that the bulk of organisms isolated from all fields and the pasture are those which constitute the smallest percentage of infectious agents, or which are not considered to be secondary pathogens (almost three-quarters if Citrobacter is excluded). The presence of Escherichia coli from the control field and the pasture and Proteus/Providencia from the pasture indicates that these areas are more likely to be health risks than are the aged sludged fields. Unless capable of growth/maintenance in the soil, these species are probably contributed by wild animals. The sludged fields are not immune to contamination by wild animals as small numbers of rodents have been noted from those locations, but deer droppings have not been seen; the latter are quite common from both the control and the pasture.

7.2.5 Total Numbers of the Enterobacteriaceae: Allergens

Regarding the numbers of the Enterobacteriaceae (which can act as allergens), there appears to be little difference between the sludged and control fields and the pasture, though the numbers from the control field are consistently fewer. The total numbers at each location fluctuate radically with the season with small numbers in the winter being supplanted by very large numbers in the spring (when cultivation and planting occur), and substantially lesser numbers being recorded in the summer and the fall (when cultivation occurs again). There is little difference within each season between the various fields and spring cultivation would contribute the largest numbers of potential allergens regardless on any field. Of course, during the spring, the soil is moist and air borne particles would be minimal.

7.2.6 The Primary Pathogens

Salmonella or Staphylococcus were not isolated from either the fields or the pasture. Acid-fast bacteria were found erratically in mixed culture from all areas except for the control field. We recorded only the presence of Mycobacterium but, because of fear of laboratory contamination, did not attempt to re-isolate the contaminated cultures for further identification. The World Health Organization (1981) indicates that, because of the virtual eradication of tuberculosis from the developed nations, transmission from sludge is no longer of significance. Of course, mycobacteria aside from those causing tuberculosis are normal inhabitants of the soil and some have been incriminated as producing various infections in the human host (e.g., Damsker and Bottone 1980).

7.2.7 Fluorescent Pseudomonas

These organisms were found upon three occasions: the 1980 and 1981 fields and the pasture during the fall samplings. The literature generally indicates that *P. aeruginosa* is ubiquitous though controversy exists.

7.2.8 The Viruses

Viruses were not isolated from the fields or the pasture. It may be assumed that these entities were not present or, if present, were not detected by the methods employed.

7.2.9 Other Organisms

We have not investigated certain organisms which can be found in sludge and which, under appropriate circumstances, can be responsible for infection. The World Health Organization (1981) refers to certain of these and its remarks are noted below:

1. *Clostridium* spp. These are soil organisms and there is no specific risk to health from sludge though sludge application can increase the level of spores in the soil.

2. *Yersinia*. This is associated with food poisoning. Further work is necessary to ascertain if sludge is an agent for infection via raw vegetables.

3. *Listeria*. Its significance in soil is not known though sludge application will add to its load in the environment. Its incidence is not of great concern.

4. Leptospira. There is no evidence that sludge is an agent of infection.

5. Campylobacter. Sewage sludge is unlikely to be involved in environmental spread or in the incidence of animal infection.

6. The Fungi. Fungi are ubiquitous in soil, harvested grains, and animal feeds. Sludge pasteurization is pointless as recontamination will occur. Main human hazards are from airborne spores from composting or sludge drying plants.

7. Taenia saginata. Of little significance in aged sludge soil mixtures. Sludge containing the eggs of this tapeworm becomes non-infective for test calves 17 to 18 weeks after sludge application.

In the Shepard area, it seems from the data presented above (both experimental and published) that surface sludged fields approach background (control) levels with regard to parasite stages, bacteria, and viruses in as little as nine months. It is likewise evident that some of the total coliforms (Citrobacter freundii and Enterobacter aerogenes) are capable of reproduction in soil when conditions are favourable.

7.3 RAW, MESOPHILIC DIGESTER, AND LAGOON SLUDGE

The numbers of parasite eggs from raw sludge averaged about 2500/dry wt. kg while from digester sludge the number was some 12 000 eggs/dry wt. kg. Theoretically, the number found in digester sludge should have been doubled instead of more than quadrupled from that noted from raw sludge; presumably, the settling of eggs in the digester and their concentration at the point of sampling could have accounted for this discrepancy. The numbers of eggs found in lagoon sludge were similar to that found from digester sludge; comparison of solid contents of digester and lagoon sludge indicates that the latter should have approximately four times as many as the former. The explanation for the discrepancy may be that, even though eggs remain in a static state in sludge, many of them are destroyed over time.

As anticipated, the numbers of secondary pathogens (Enterobacteriaceae) and total coliforms isolated from raw sludge are large. Additionally, the percentages of total coliforms are high. The efficiency of anaerobic mesophilic digestion upon the reduction of the total numbers was found to be (for the Fish Creek facility only) 89.8% and 99.1%, the former figure being somewhat low according to published information. Even though the numbers of the organisms were greatly reduced, it is interesting to note that the relative proportion of the species remaining in the digester sludge were similar to those found from the raw sludge; likewise, the relative percentages of total coliforms are similar. Digestion, then, is efficient in reducing the numbers of the Enterobacteriaceae but has no selective action upon the species present nor upon the percentage of total coliforms (i.e., the members of the Enterobacteriaceae respond similarly to the effects of digestion).

Insofar as primary pathogens are concerned, coagulase positive staphylococci and Salmonella were isolated from plant sludge. These organisms were not found later from either lagoon sludge or sludged fields. These findings support other data which indicate that most primary pathogens have a limited existence in lagoon and applied sludges.

Regarding the secondary pathogens, E. coli was routinely isolated from all sludges while Proteus/Providencia were found from all sludge types though not with the frequency noted for E. coli. The percentage of the combined numbers of E. coli and Proteus/Providencia averages 62.7% of the populations of all sludges,a figure that compares favourably with the figure of 66.3% given by the Center for Communicable Disease as the percentage of infections attributed to E. coli and Proteus/Providencia.

When lagoon sludge is deposited on land, E. coli remains in analytical view for a period of time,exceeding that noted for Proteus/Providencia. Enterobacter/Serratia, which constitute the smallest percentage of CDC-reported infective agents averaged 18.5% of the microbial constituency of the sludges as compared with the 58.5% of such organisms reported from sludged/control fields and pasture.

The TNTC (too numerous to count) notation given to the MacConkey positive forms from lagoon sludge indicates that these organisms are capable of growth in the sludge lagoons, growth that is to a point higher than noted from raw sludge. The data (Table 23) also indicate that the relative proportions of the species of the Enterobacteriaceae and the percentage of total coliforms from lagoon sludge is similar to that found from raw and digester sludge. It is only after placement on land that the proportions of species deviate. It is presumed that this deviation is because certain species (notably *E. coli* and *Proteus/Providencia*) are more susceptible to the conditions found in the soil ecosystem and are unable to survive for extended periods of time on land. Others of the Enterobacteriaceae (e.g., *Enterobacter*, *Serratia*, *Hafnia*), present in lesser numbers or analytically undetectable from sludges find conditions on land more suitable for their survival and reproduction, and hence the appearance of overgrowth. The primary pathogens (e.g., coagulase positive staphylococci and *Salmonella*), do not display this cyclic development: digestion reduces the numbers found in raw sludge and further reduction occurs in sludge lagoons. Any residual population is eventually (depending upon local conditions) eliminated when lagoon sludge is placed on land.

7.4 BURIED/INJECTED SLUDGE

Hand-buried sludge showed large numbers of parasite eggs (17 000/dry wt. kg) at 0-time but, after remaining undisturbed for 258 days, the number had dropped to 220/dry wt. kg. Machine injected sludge, examined seven days after deposition showed some 10 000 eggs/dry wt. kg; after discing, only the top portion of the field was examined and 66 days after injection and 55 days after discing, no eggs were recovered. It seems evident that sludge buried and left undisturbed does not contribute to the maintenance of parasite eggs, possibly due to the predation by fungi and soil invertebrates. Injected sludge with its contained parasite stages brought to the surface by discing and allowed to remain at or near the surface of a fallow field experiences the same deleterious environmental influences (i.e., high temperatures and low moisture content), as were noted in the 1981 surface sludged field, with a resultant destruction of parasite stages; presumably that portion of the injected sludge not brought to the surface would be exposed to the same parasiticidal features of hand-buried sludge with subsequent destruction of the stages.

Complete bacteriological studies were not performed on the 0-time _hand-buried_ sludge but, after 258 days, small numbers of Enterobacteriaceae (<2000/dry wt. g) were found along with lesser numbers of total coliforms (1000×10^4/dry wt. g). The total coliforms constituted 55% of the organisms, a figure exceeding the 1980 and 1981 sludged fields by about two times, and comparable to the percentages noted in digester sludge; total numbers were, of course, reduced. _Proteus/Providencia_ were not found but _E. coli_ were recovered at a rate of 267×10^4/dry wt. g. The implication of these data is that, while the numbers of Enterobacteriaceae and total coliforms are reduced during prolonged burying, _E. coli_ are present which can contaminate the surface if subsequent cultivation disturbs the sludge to the surface.

With regard to _injected sludge_, the first of two discings exposed the bacteria surfaced to the negative deterrents of the topsoil (high temperature and low moisture); in addition, various microbial interactions were in effect. By 55 days, the MacConkey positive organisms and the total coliforms were similar in numbers to those found from the sludged and control fields. _Proteus/Providencia_ were not found but low numbers of _E. coli_ (5×10^4/dry wt. g) were noted.

Following the second discing, the soil was recontaminated with sludge and Enterobacteriaceae were recovered but at a level three to four orders of magnitude less than those noted from the original injection. The percentages of total coliforms were initially high, though the numbers were lesser than those from the original material. Fifty-nine days after the second discing, the field resembled the control and surface sludged fields. _Proteus/Providencia_ and _E. coli_ were never isolated.

If, after the first discing, the field had remained untouched, the _E. coli_ would presumably have disappeared over time and the field would have achieved background level. The second discing, of course, recontaminated the field insofar as the percentages of total coliforms are concerned (though the numbers were small) but these diminished over time. We have no data to present as to what would have occurred had the second discing been delayed until spring. We presume, though, that the influence of inter-microbial activity upon the Enterobacteriaceae in the _fragmented_ sludge would have reduced both the numbers of MacConkey positive forms and the percentage of total coliforms to an acceptable (background) level.

8. CONCLUSIONS

1. The source of most parasite stages in sewage, and ultimately sludge, is from domestic animals.

2. Most parasite stages are found in sludge;few are found in effluent.

3. The cysts of protozoan parasites are adversely affected by anaerobic, mesophilic digestion. The eggs of parasitic worms are not greatly influenced by digestion. In lagoon sludge there is attrition of worm eggs but the survivors remain static until exposed to air.

4. Parasite stages at or near the surface die within weeks in surface sludged, disced soil when the field is allowed to remain fallow during the warmer months. Those worm eggs carried by discing to intermediate and lower levels (5 to 20 cm) by discing reach low levels within months and will disappear in months to years. Recontamination of the surface layer (0 to 2.5 cm) by subsequent discing will result in destruction of eggs within weeks during the warmer months.

5. Parasite stages in buried sludge are reduced to small numbers within nine months if the sludge is undisturbed. Fragmentation of sludge pockets by discing results in destruction of worm eggs within months.

6. Parasite stages in injected and disced sludged soil are killed within weeks in the top 2.5 cm if the field lies fallow during the warm months.

7. The numbers of the Enterobacteriaceae in raw sludge are reduced by 90 to 99% during anaerobic, mesophilic digestion.

8. After deposition in sludge lagoons, the numbers of the Enterobacteriaceae increase in numbers to levels exceeding those found in raw sludge.

9. The proportions of the genera of the Enterobacteriaceae, and the percentages of total coliforms are similar in raw, digester, and lagoon sludges.

10. Primary pathogens are reduced sequentially in numbers through anaerobic digestion and lagoon storage, and following the application of sludge to land.

11. After deposition of sludge on land, the secondary pathogens (Enterobacteriaceae) producing the greatest number of recorded infections (*Escherichia coli* and *Proteus/Providencia*) are rapidly reduced in numbers and/or destroyed. Other Enterobacteriaceae producing the smallest number of infections (*Enterobacter* and *Serratia*) or not recorded as being of appreciable significance (*Hafnia*) become paramount and represent the largest percentage of the species encountered.

12. With the exception of *E. coli* and *Proteus/Providencia*, the members of the Enterobacteriaceae (excluding *Yersinia* for which no data are available) are capable of growth on sludged land, unsludged land, and pasture.

13. The members of the total coliforms (except *E. coli*) can grow on sludged land, unsludged land, and pasture.

14. Faecal coliforms (MPN) appear erratically over long periods on sludged soil; however, they disappear from analytical view during winter months. Faecal coliforms are likewise found erratically from grazed land though it is believed that cattle contribute the organisms. Faecal coliforms isolated from the control field are presumed to have been contributed by deer.

15. The numbers of Enterobacteriaceae and total coliforms are several orders of magnitude greater during the spring than during winter, summer, or fall, irrespective of the type of field (i.e., sludged, control, or pasture).

16. In excess of 70% of the total coliforms from mesophilic digesters or lagoon sludge are represented by *E. coli*. The

organism is not found from fields sludged in excess of eight months.

17. Small numbers of E. coli were found from a control field and larger numbers were isolated from a pasture. The source of the organism from the former is believed to be from deer while from the latter both deer and cattle are contributory.

18. Acid fast bacteria (not identified) are found sporadically from sludged fields and pasture.

19. Fluorescent (37^{o}) pseudomonads can be isolated from sludges and recently sludged fields (seven days). They have been encountered from sludged fields and the control field but only during the fall.

20. Viruses were not found from lagoon sludge, sludged fields, a control field,or a pasture.

21. Initial discing of sludge injected fields contaminated the surface with Enterobacteriaceae (except Proteus/Providencia) including total coliforms. Within days, the numbers of all species and the percentage of total coliforms drop to levels approximating the control field.

22. After a second discing of an injected field, recontamination by members of the Enterobacteriaceae (except E. coli and Proteus/Providencia) occurs but the numbers are low and similar to the control field. The percentage of total coliforms is high (comparable to the pasture) but the numbers are small and within days the percentage of total coliforms resembles the control field.

23. Escherichia coli may be isolated for long periods of time from sludge applied to grassland.

24. The percentages and numbers of total coliforms (ECE) remain high for long periods of time from sludge applied to grassland.

25. *Proteus/Providencia* do not survive for more than a few weeks from sludge applied to grassland.

26. Parasite stages disappear within a few weeks after placement of sludge on grassland.

27. After overwintering, sludge applied to grassland is similar to the control field.

9. RECOMMENDATIONS AND SUGGESTIONS

The recommendations outlined below are based upon the work done during the past three years; reinforcement of certain of the data may be found in the sections on literature review and/or citations noted in the body of the experimental data. It must be emphasized that any recommendations are restricted to the conditions found at the fields sludged in the Shepard area and at the Crossfield lagoons. Application of these recommendations elsewhere must be based upon those situations where climatic, soil, and sludge conditions are similar; it is essential to consider and assess local conditions when applying these recommendations: when they are similar, the recommendations may be put into force.

The following recommendations are with regard to biological health hazards (parasite stages, bacteria, and viruses) associated with (1) the disposal of 12-month lagoon effluent from Crossfield; and (2) the disposal of anaerobic, mesophilically digested sludge from Bonnybrook/Fish Creek on agricultural land. Obviously no cognizance is taken of the levels of nitrogen or phorphorus, trace elements, or organic compounds; it is presumed that engineering practice will denote acceptable levels of these prior to sludge application.

9.1 RECOMMENDATIONS

1. Effluent from the 12-month lagoon at Crossfield may be used for spray irrigation of fodder crops:
   (A) one month of warm weather (July to mid-September) should elapse after spraying before animals are allowed to graze; and
   (B) If hay is to be taken from the field, one month of warm weather (July to mid-September) should intervene between spraying and harvesting.

2. Surface sludged and disced land should be allowed to remain fallow throughout the following winter after which any cereal or oil crop may be planted; animals may be allowed to graze on any plant residue following the harvest.

3. Winter cereal crops may be planted in the fall following surface sludging, discing and fallowing. Animals may consume the plant residue the following year after harvesting.

4. If market gardening (truck farming) is contemplated (i.e., leafy vegetables, root crops, cole vegetables, legumes etc.), the following regime should be followed. The field after sludging, discing, and fallowing overwinter should be planted the next spring with cereal or oil crops; the following year (two years after sludging), consumable vegetables may be planted. If fall cereal crops are planted in the same year as sludging/discing/fallowing, the crops should be harvested and the field allowed to fallow through the second winter after which consumable crops may be planted.

5. For injected sludge, the same recommendations as noted in 2, 3, and 4 should be followed.

6. If sludge is applied to pasture, it should be deposited in the spring or early summer. Animals should not be allowed to graze until the following year.

7. If the sludged land is to be used for hay and/or grazing, the soil should be disced and allowed to be fallow over winter. The crop should be planted in the spring; cutting and/or grazing is then at the discretion of the farmer.

9.2 SUGGESTIONS FOR FURTHER RESEARCH

1. Further investigations (similar to those noted in this report) should be conducted upon thermophilically digested sludge.

2. Further work should be done on the isolation of *Salmonella* from all systems, including soil. These investigations should be concerned with the resurrection of damaged cells, and should compare the effectiveness of different types of selective incubation media.

10. REFERENCES CITED

Alba, S. and R. Sudo. 1964. Discussion following paper by: H. Liebmann, 1964. Parasites in sewage and the possibilities of their extinction. Adv. Water Poll. Res. 2:282-284.

Akin, E.A. et al. 1978. Health hazards associated with wastewater effluents and sludges: microbiological considerations, In Proceedings of the Conference on Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges, ed. Sagik, B.P. and C.A. Sorber. Dec. 12-14, 1977; The University of Texas at San Antonio. 329 pp.

Alberta Environment. 1977. A practical guide to municipal wastewater irrigation: discussion and guidelines. Edmonton: Earth Sci. Licens. Div. 31 pp.

Almasi, P. et al. 1971. Decontamination of solid and liquid urban wastes by composting. Hyg. Sanit. 36:438-441.

Bausum, H.T. et al. 1976. Bacterial aerosols resulting from spray irrigation with waste water. U.S. Army Med. Bioeng. Res. Dev. Lab., AD/A-02P 359.

Beard, P.J. 1938. The survival of typhoid in nature. J. Amer. Water Works Assoc. 30:124.

Beard, P.J. 1940. Longevity of Eberthella typhosus, in various soils. Amer. J. Pub. Health 30:1077.

Beaver, P.C. 1975. Biology of soil transmitted helminths: the "massive" infection. Health Lab. Sci. 12:116-125.

Beck, A. 1979. The impact of the canine clean-up law. Environ. 21:28-31.

Bell, R.G. 1976. Resistance of fecal coliform indicator bacteria on alfalfa irrigated with municipal sewage lagoon effluent. J. Environ. Quality. 5:39-42.

Bhaskarian, T.R. et al. 1956. Studies on the effect of sewage treatment processes on the survival of intestinal parasites. Ind. J. Med. Res. 44:163-180.

Bissonnette, G.K. et al. 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Microbiol. 29:186-194.

Bissonnette, G.K. et al. 1977. Evaluation of recovery methods to detect coliforms in water. Appl. Environ. Microbiol. 33:590-595.

Boyd, J.W. et al. 1969. Bacterial response to the soil environment. Colorado State University Sanitary Engineering Papers.

Bonde, G.J. 1963. Bacterial Indicators of Water Pollution, 2nd ed. Copenhagen: Teknisk Forlag.

Bowmer, E.J. 1973. Parasites galore: intestinal parasites in British Columbia 1956-1972 (17 years). Can. J. Pub. Health 64:83-92.

Bringman, G. and R. Kuhn. 1959. Viability of coli bacteria on plants in the open in relation to time and weather. Gesundheitsung 79:1-8.

Bringman, G. and G. Trolldenier. 1960. Experiments on the survival of coliform organisms on forage plants watered with sewage. Bull. Hyg. 35:221.

Brouwer, H., J.C. Lance, and M.S. Riggs. 1974. High rate land treatment. II: Water quality and economic aspects of Flushing Meadows Project. J. Water Poll. Control Fed. 46:844-851.

Bryan, F.L. 1974. Diseases transmitted by foods contaminated with wastewater. Proc. Conf. Use. Wastewater Prod. Food Fiber, Oklahoma City, 1973.

Buchanan, R.E. and N.E. Gibbons. 1974. Bergey's manual of determinative bacteriology, 8th ed. Williams and Wilkins, Baltimore. 1268 pp.

Burge, W.D. and P.D. Millner. 1980. Health aspects of composting: primary and secondary pathogens. In Sludge - health risks of land application, ed. G. Bitton et al. Ann Arbor Science Publishers Inc., Ann Arbor, Michigan.

Canadian Wildlife Federation. 1977. Treated raw sewage being used to irrigate crops. Environment, International Wildlfe. Nov.-Dec., pp. 320-321.

Carroll, T.E. et al. 1975. Review of landspreading of liquid municipal sewage sludge. Battelle Columbus Laboratories. U.S. Environmental Protection Agency Report No. EPA/670/2-75/059-F.

Carson, L.A., N.J. Petersen, M.S. Favero, and S.M. Aguero. 1978. Growth characteristics of atypical Mycobacteria in water and their comparative resistance to disinfectants. Appl. Environ. Microbiol. 36:839-846.

Center for Disease Control. 1978. National nosocomial infections study report, annual summary 1976. Center for Disease Control, Atlanta, Georgia.

Chamberlin, C.E. and R. Mitchell. 1978. A decay model for enteric bacteria in natural waters. In Water Pollution Microbiology, Vol. II, ed. R. Mitchell. New York, Wiley.

Chandler, A.S. and C.P. Read. 1961. Introduction to Parasitology, 19th ed. New York, Wiley.

Chang, S.I. et al. 1960. Survival and protection against chlorination of human enteric pathogens in free-living nematodes isolated from water supplies. Amer. J. Trop. Med. Hyg. 9:136-142.

Christman, R.F. 1972. Assessment of the effectiveness and effects of land disposal methodologies of wastewater management. U.S. Army Corps of Engineers.

Clark, C.S. et al. 1981. Evaluation and the health risks associated with the treatment and disposal of municipal wastewater and sludge. U.S. Environmental Protection Agency, Cincinnati, Report No. R805445.

Cliver, D.O. 1980. Agricultural crops: pathogens. In Sludge health risks of application. ed. G. Bitton et al. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Cody, R.M. and R.G. Tischer. 1973. Isolation and frequency of occurrence of *Salmonella* and *Shigella* in stabilization ponds. J. Water Poll. Cont. Fed. 37:1399-1403.

Collins, C.H. and P.M. Lyne. 1976. Microbiological Methods, 4th ed. Butterworths.

Colwell, R.R., B. Austin, and L. Wan. 1978. Public Health Considerations of the microbiology of "potable water." In Evaluation of the microbiology standards for drinking water, ed. C.W. Hendricks, U.S. Environmental Protection Agency, Report No. EPA570/9-78-002. Washington, D.C.

Cooper, R.C. et al. 1974. Effect of disposable diapers on the composition of leachate from a landfill. Sanit. Engin. Res. Lab., Coll. Engin. Sch. Pub. Health, U. of Calif., Berkeley. Series Report No. 74-3.

Cram, E.B. 1943. The effect of various treatment processes on the survival of helminth ova and protozoan cysts and sewage. Sew. Works. J. 15:1119-1138.

Crewe, W. and R. Owen. 1978. 750 000 eggs a day - £750 000 a year. New Scientist 78:344-346.

Damsker, B. and E.J. Bottone. 1980. Non-tuberculosis mycobacteria as unsuspected agents of dermatological infections: diagnosis through microbiological parameters. J. Clin. Microbiol. 11:569-572.

Dazzo, F., P. Smith, and D. Hubbell. 1972. Vertical dispersal of fecal coliforms in Scranton fine sand. Proc. Crop. Sci. Soc. Florida 32:99-102.

Dedie, K. 1955. Sewage organisms pathogenic to animals. Stadtehyg. 177-180.

Doran, J.W., J.R. Ellia, and T.M. McCalla. 1976. Microbial concerns when wastes are applied to land. In Land as a waste management alternative. ed. A. Loehr. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Drurie, P.H. 1961. Parasitic gastro-enteritis of cattle: the distribution and survival of infective strongyle larvae on pasture. Austrl. J. Agricult. 12:1200-1211.

Dudley, D.J. et al. 1980. Enumeration of potentially pathogenic bacteria from sewage sludges. Appl. Environ. Microbiol. 39:118-126.

Dufour, A.P. 1977. Escherichia coli: the fecal coliform. In Bacterial indicators/health hazards associated with water, ed. A.W. Hoadley and B.J. Dutka. Am. Soc. Testing and Materials Special Technical Publication No. 635, Philadelphia, Penn. 356 pp.

Dunlop, S.G. 1968. Survival of pathogens and related disease hazards. Chap. IX in Municipal sewage effluent for irrigation, ed. Wilson and Beckett. Louisiana Tech. Alumni Foundations, pp. 107-122.

Dutka, B.J., Kuchma, S. and K.K. Kwan. 1979. Fecal coliform and E. coli estimates, tip of the iceberg. Water, Air and Soil Pollution 11:349-362.

Dutka, B.J. and S.E. Tobin. 1976. Study on the efficiency of four procedures of enumerating coliforms in water. Can. J. Microbiol. 22:630-635.

ECO/LOG Week. 1978. Seminar updates sewage sludge use and disposal. February 17, 1978.

Edds, G.T. and J.M. Davidson. 1981. Sewage sludge - viral and pathogenic agents in soil-plant-animal systems. U.S. Environmental Protection Agency, Cincinnati, Report No. EPA-600/1-81-026.

Edmonds, R.L. 1976. Survival of coliform bacteria in sewage sludge applied to a forest clearcut and potential movement into groundwater. Appl. Environ. Microbiol. 32:537.

Edmonds, R.L. and W. Littke. 1978. Coliform aerosols generated from the surface of dewatered sewage applied to a forest clearcut. Appl. Environ. Micro. 36:972-974.

Eller, C. and F.F. Edwards. 1968. Nitrogen-deficient medium in the differential isolation of Klebsiella and Enterobacter from feces. Appl. Microbiol. 16:896-899.

Elliott, L.F. and J.R. Ellis. 1977. Bacterial and viral pathogens associated with land application of organic wastes. J. Environ. Quality 6:245.

Evans, J.B. 1976. Coagulase positive staphylococci as indicators of potential health hazards from water. In Bacterial Indicators/health hazards associated with water, ed. A.W. Hoadley and B.J. Dutka. Am. Soc. for Testing and Materials, Special Technical Publication No. 635. 356 pp.

Evans, T.M. 1975. The limulus lysate assay: a rapid and sensitive test of bacterial water quality. Bozeman MON: Montana State University. M.Sc. Thesis.

Ewing, B.B. and R.J. Dick. 1970. Disposal of sludge on land, water quality improvement by physical and chemical processes. U. of Texas Press, Austin.

Ewing, W.H. and W.J. Martin. 1974. Enterobacteriaceae. In Lennette and Spaulding "Manual of Clinical Microbiology." 2nd ed. Am. Soc. Microbiol., Bethesda.

Farmer, J.J. and D.J. Brenner. 1977. Concept of a bacterial species: Importance to writers of microbiological standards for water. In Bacterial indicators/health hazards associated with water, ed. A.W. Hoadley and B.J. Dutka. Am. Soc. Testing and Materials, Special Technical Publication No. 635. 356 pp.

Faust, E.C. et al. 1938. A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. Am. J. Trop. Med. Hyg. 18:169-183.

Faust, E.C. et al. 1939. Comparative efficiency of various technics for the diagnosis of protozoan and helminths in feces. J. Parasitol. 25:241-262.

Faust, E.C. and P.F. Russell. 1964. Clinical Parasitology, 7th ed. Lea and Febiger.

Findlay, C.R. 1973. Salmonella in sewage sludge: Part II. Multiplication Vet. Rec. 93:102-103.

Fitzgerald, P.R. 1976. Recovery and utilization of strip-mined land by application of anaerobically digested sludge and wildlife grazing. Ann Arbor Sci. Pub., pp. 497-505.

Fitzgerald, P.R. 1979. Potential impact on the public health due to parasites in soil sludge systems. Proc. 8th Nat. Conf. Munic. Sludge Management. Miami Beach FL. pp. 214-221.

Fitzgerald, P. 1981. Helminths and heavy metals transmission from anaerobically digested sewage sludge. U.S. Environmental Protection Agency, Mun. Env. Res. Lab., Cincinnati. EPA-600/S2-81-024.

Fitzgerald, P.R. and R.F. Ashley. 1977. Different survival of Ascaris ova in wastewater sludge. J. Water Poll. Con. Fed. 49:1722-1744.

Fitzgerald, P.R. and M.E. Mansfield. 1970. Visceral larval migrans (Toxocara canis) in calves. Am. J. Vet. Res. 31:561-565.

Fitzgerald, P.R. and T.B.S. Prakasam. 1978. Survival of Trichinella spiralis larvae in sewage sludge anaerobic digesters. J. Parasit. 64:445-447.

Forstner, M.J. 1970. The effect of sewage, overlying liquor and composting on the viability of parasitic reproductive stages. Z. Wass. Abwass. Forsch. 4:180.

Foster, D.H. and R.S. Engelbrecht. 1973. Microbial hazards in disposing of wastewater on soil. In Recycling treated municipal wastewater and sludge through forest and cropland, ed. W.E. Sopper and L.T. Kardos. Penn. State University Press.

Fox, J.C. and P.R. Fitzgerald. 1976. Parasitic organisms present in sewage systems of a large sewage district. J. Parasit. 62 (Suppl.):28.

Fox, J.C. and P.R. Fitzgerald. 1978. The presence of Giardia lamblia cysts in sewage sludge from the Chicago area. Paper presented at Conference: Waterborne Transmission of Giardiasis. Cincinnati, 1977.

Gaby, W.L. 1975. Evaluation of health hazards associated with solid waste/sewage sludge mixtures. U.S. Environmental Protection Agency, Cincinnati, Report No. EPA-670/2-75-023.

Galton, M.M. et al. 1954. *Salmonella* in swine, cattle, and the environment of abbatoirs. J. Infect. Dis. 95:236-245.

Geldreicht, E.E., M.J. Allen, and R.H. Taylor. 1978. Interferences to coliform detection in potable water supplies. *In* Evaluation of the microbiology standards for drinking Water. U.S. Environmental Protection Agency, Washington, D.C., C.W. Hendricks ed., EPA-570/9-78-002.

Georgi, J.R. 1969. Parasitology for Veterinarians. Saunders.

Gerba, C.P., C. Wallis, and J.L. Melnick. 1975. Fate of wastewater bacteria and viruses in soil. J. Irrig. Drainage Div. ASCE, 3:157.

Glantz, P. and T. Jacks. 1967. Significance of *Escherichia coli* serotypes in wastewater effluent. J. Water Poll. Control Fed. 39:1918.

Goleuke, C.G. 1972. Composting — a study of the process and its principles. Rodale Press, Emmaus, Pa.

Gotass, H.B. 1956. Composting - sanitary disposal and reclamation of organic wastes. WHO Monograph. Series No. 31.

Grabow, W.O.K. and E.M. Nupen. 1972. The load of infectious microorganisms in the wastewater of two South African hospitals. Water Research 6:1557-1563.

Grabow, W.O.K. and M. du Preeze. 1979. Comparison of m-Endo LES, MacConkey, and Teepol media for membrane filtration counting of total coliform bacteria in water. Appl. Environ. Microbiol. 38:351-358.

Graham, H.J. 1981. Parasites and the land application of sewage sludge. Ontario Ministry of the Environment. Project Number 74-1-41.

Greenburg, A.E. and B.H. Dean. 1958. The beef tapeworm, measly beef and sewage -- a review. Sew. Ind. Wastes 30:262-269.

Greenberg, A.E. and E. Kupta. 1957. Tuberculosis transmission by wastewater - a review. Sew. Ind. Wastes 5:524-537.

Grieg-Smith, P. 1964. Quantitative plant ecology. Plenum Press.

Gritzuk, M. 1973. Ocean County sewage authority wastewater solids and utilization on land demonstration project. In Proceedings and Conference on Land Disposal of Municipal Effluents and Sludges held at Rutgers, The State University of New Jersey; March 12-13, 1973. U.S. Environmental Protection Agency, Report No. PB-227-115.

Guentzel, M.N. 1977. Potential impact of water sources of bacterial pathogens in wastewater applied to land. In Risk assessment and health effects of land application of unicipal wastewater and sludges, ed. B.P. Sagik and C.A. Sorber. U. of Texas, San Antonio.

Guentzel, M.N. 1980. Escherichia, Klebsiella, Enterobacter, Serratia and Citrobacter in community and hospital-acquired (nosocomial) infections. In Microbiology principals and practice, ed. S. Baron. Addison-Wesley, Menlo Park, California.

Guthrie, R.K., D.S. Cherry, and F.L. Singleton. 1976. Alteration of microbial populations in thermal stress. J. Water Poll. Control Fed. 48:962-965.

Hanks, T.G. 1967. Solid waste/disease relationships. A literature survey. National Center for Urban and Industrial Health Solid Wastes Program, Bureau of Disease Prevention and Environmental Control. Cincinnati.

Harbourne, J.F. 1978. Salmonella in effluent waters and river sites in North Yorkshire. Brit. Vet. J. 134:350-357.

Harding, H.J., Thomas, R.E., Johnson, D.E. and C.A. Sorber. 1981. Aerosols generated by liquid sludge application to land. U.S. Environmental Protection Agency, Cincinnati, Report No. EPA 600/1-81-028.

Hays, B.D. 1977. Potential for parasitic disease transmission with land application of sewage plant effluents and sludges. Water Research 11:583-595.

Healy, G. and G.S. Visvesvara. 1977. Obligate parasites and other opportunistic organisms in the water resource. In Risk assessment and health effects of land application of municipal wastewater and sludges ed. B.P. Sagik and C.A. Sorber. U. of Texas, San Antonio.

Hess, E. and C. Breer. 1975. Epidemiology of Salmonella and fertilizing of grassland with sewage sludge. Zbl. Bakt. Parasit. Infeckt. Hyg. Abstract 1, 161:154-160.

Heukelekian, H. and M. Albanese. 1956. Enumeration and survival of human tubercle bacilli in polluted waters. Vol. II: Effect of sewage treatment and natural purification. Sew. Ind. Wastes 28:1094-1102.

Hinsely, T.D. et al. 1972. Agricultural benefits and environmental changes resulting from the use of digested sludge on field crops. U. of Illinois Press.

Hoadley, A.W. and C.M. Cheng. 1974. Recovery of indicator bacteria on selective media. J. Appl. Bacteriol. 37:45-57.

Hoadley, A.W. and S.M. Goyal. 1976. Public health implications of the application of wastewaters to land. In Land treatment and disposal of municipal and industrial wastewater, ed. R.L. Sanks and T. Asano. Ann Arbor Science, Ann Arbor, Michigan.

Hoadley, A.W. 1977. Potential health hazards associated with Pseudomonas aeruginosa in water. In Bacterial indicators/health hazards associated with water, ed. A.W. Hoadley and B.J. Dutka. Am. Soc. for Testing and Materials, Special Technical Publication No. 635, Philadelphia, Pa. 356 pp.

Hugh, R. and G.L. Gilardi. 1974. *Pseudomonas*. *In* Manual of clinical microbiology, ed. Lennette, Spaulding and Truant, 2nd ed. A. Soc. Microbiology, Washington, D.C.

Hutchinson, D., R.H. Weaver, and M. Scherago. 1943. The incidence and significance of microorganisms antagonistic to *Escherichia coli* in water. J. Bact. 45:29.

Hyde, H.C. 1976. Utilization of wastewater sludge for agricultural soil enrichment. J. Water Poll. Control Fed. 48:77.

Iwanszuk, J. and W. Dozanska. 1956. Effects of chlorination on the survival of *Ascaris suis* eggs in sewage. Water Pollution Abstract No. 2074.

Jack, E.J. 1969. An outbreak of *Salmonella typhimurium* infection in cattle associated with the spreading of slurry. Vet. Res. 84:196-199.

Jackson, G.J., Bier, J.W. and R.A. Rude. 1977. Recycling of refuse into the food chain: the parasite problem. *In* Risk assessment and health effects of land application of municipal wastewater and sludges, ed. B.P. Sagik and C.A. Sorber. U. of Texas, San Antonio.

Jannock, G.W. 1971. Studies on the survival of *Salmonella typhimurium* and *Salmonella bovimorbificans* on pasture and in water. Australian Vet. Journal 47:557-559.

Jensen, K.E. 1954. Presence and destruction of tubercle bacteria in sewage. Bull. World Health Organization 10:171-179.

Johnson, P.E. et al. 1977. Aerosol monitoring for microbial organisms near a spray irrigation site. In Risk assessment and health effects of land application of municipal wastewater and Sludges, ed. B.P. Sagik and C.A. Sorber. U. of Texas, San Antonio.

Jolley, W.R. and P.R. Fitzgerald. 1976. Sludge in pasture. Illinois Res. 18:10-11.

Jones, A.E., W. Crewe, and R.R. Owen. 1979. Enhanced survival of eggs of Ascaris following ingestion by earthworms. Trans. Royal Soc. Trop. Med. Hyg. 73:325.

Kabler, P. 1959. Removal of pathogenic microorganisms by sewage treatment processes. Sew. Ind. Wastes 31:1373-1382.

Kates, K.C. 1950. Survival on pasture of free-living stages of some common gastro-intestinal nematodes of sheep. Proc. Helminth. Soc. Washington 17:39-58.

Katzenelson, E., I. Bulum, and H.I. Shuval. 1976. Risks of communicable disease infection associated with wastewater irrigation in agricultural settlements. Science 194:944-946.

Kawata, K., W.N. Cramer, and W.D. Burge. 1977. Composting destroys pathogens. Water Sew. Works 124:76-79.

Kebina, V.Y. and G.L. Ploscheva. 1974. Sanitary helminthology evaluation of the waste water treatment method of a small industrial sewage plant. Gig. Sanit. 7:93.

Keller, P. 1951a. Sterilization of sewage sludges: I: a review of the literature pertaining to the occurrence and viability of parasitic ova and cysts in sewage with special reference to Ascaris lumbricoides. J. Proc. Inst. Sew. Purif. Pt. 1:92-99.

Keller, P. 1951b. The influence of heat treatment on the ova of *Ascaris lumbricoides* in sewage. J. Proc. Inst. Sew. Purif. Pt. 1:100-109.

Kenner, B.A., G.K. Dotson, and J.E. Smith. 1971. Simultaneous quantification of *Salmonella* species and *Pseudomonas aeruginosa*. U.S. Environmental Protection Agency, Nat. Env. Research Center, Cincinnati, Ohio, Report No. PB-213-706.

Kott, H. and Y. Kott. 1967. Detection and viability of *Endamoeba histolytica* cysts in sewage effluents. Water Sewage Works 114:117-180.

Kott, Y. 1973. Hazards associated with the use of chlorinated oxidation pond effluents for irrigation. Water Res. 7:853-862.

Kott, Y. 1977. Current concepts of indicator bacteria. *In* Bacterial indicators/health hazards associated with water, ed. A.W. Hoadley and B.P. Dutka. Am. Soc. Testing and Materials, Special Technical Publication No. 635, Philadelphia, Pa. 356 pp.

Krasnonos, L.I. 1978. Many-year viability of *Ascaris* eggs (*Ascaris lumbricoides*) in the soils of Samarkand. Medsk. Parazit. 47:103-106. (Abstract in Trop. Dis. Bull. 75:991-992.)

Krige, P.R. 1964. A survey of the pathogenic organisms and helminthic ova in composts and sewage sludge. J. Proc. Inst. Sew. Purif. 215-220.

Krishnaswami, S.W. and F.J. Post. 1968. Effect of chlorine on *Ascaris* (Nematoda) eggs. Health Lab. Sci. 55:225-227.

Krone, R.B. 1968. The movement of disease producing organisms through soils. *In* Municipal sewage effluent for irrigation, ed. Wilson and Bennets. Louisiana Polytechnical Institute.

Kruse, H. 1962. Some present day sewage treatment methods in use for small communities in the Federal Republic of Germany. WHO Bull. 26:542.

Laird, M. et al. 1980. Intestinal Parasites of Man in the Canadian Environment. National Research Council. Canada. Assoc. Comm. Scient. Criteria Env. Quality. 1-69.

Larkin, E.P. et al. 1977. Land application of sewage wastes: potential for contamination of foodstuffs and agricultural soils by viruses and bacterial pathogens. In Risk assessment and health effects of land application of municipal wastewater and sludges, ed. B.P. Sagik and C.W. Sorber. Cent. Appl. Res. Technol., U. of Texas, San Antonio.

Leclerc, H. et al. 1977. Microbiological monitoring -- A new test for fecal contamination. In Bacterial indicators/health hazards associated with water, ed. A.W. Hoadley and B.J. Dutka. Am. Soc. Testing and Materials, Special Technical Publication No. 635, Philadelphia, Pa. 356 pp.

Lehmann, D.L. et al. 1981. Potential health problems caused by the disposal of treated sewage products containing parasites. First Annual Report to Alberta Environment from the Kananaskis Centre for Environmental Research, University of Calgary.

Lehmann, D.L. et al. 1982. Potential Health Problems Caused by the Disposal of Treated Sewage Products Containing Parasites. Second Annual Report to Alberta Environment from the Kananaskis Centre for Environmental Research, University of Calgary.

Levin, M.A. 1977. Bifidobacteria as water quality indicators. In Bacterial indicators/health hazards associated with water, ed. A.W. Hoadley and B.P. Dutka. Am. Soc. for Testing and Materials, Special Technical Publication No. 635, Philadelphia, Pa. 356 pp.

Levine, N.D. 1968. Nematode parasites of domestic animals and man. Burgess.

Liebmann, H. 1964. Parasites in sewage and the possibilities of their extinction. Adv. Water Poll. Res., 2:269-276.

Little, M.D. 1980. Agents of health significance. *In* Sludge - health risks of land application, ed. G. Bitton et al. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Lundholm, R. and R. Rylander. 1980. Occupational symptoms among compost workers. J. Occupat. Med. 22:256-257.

Mattsby, I. and R. Rylander. 1978. Clinical and immunological findings in workers exposed to sewage dust. J. Occupat. Med. 20:690-692.

Maxey, R.B. 1970. Non-lethal injury and limitations of recovery of coliform organisms on selective media. J. Milk Food Technol. 33:445-448.

McCambridge, J. and T.A. McMeekin. 1981. Effect of solar radiation and predacious microorganisms on survival of fecal and other bacteria. Appl. Env. Microbiol. 41:1083-1087.

McCullough, N.B. and W. Eisele. 1951a. Experimental human Salmonellosis. I: Pathogenicity of strains of *S. meleagridis* and *S. anatum* obtained from spray-dried whole eggs. J. Infect. Dis. 88:278.

McCullough, N.B. and W. Eisele. 1951b. Experimental human Salmonellosis. III: Pathogenicity of strains of *Salmonella newport*, *S. derby* and *S. bareilly* obtained from spray-dried whole eggs. J. Infect. Dis. 89:209.

McCullough, N.B. and W. Eisele. 1951c. Experimental human salmonellosis. IV: Pathogenicity of strains of *Salmonella pulorum* obtained from spray-dried whole eggs. J. Infect. Dis. 89:250.

McDonald, J.D. 1920. Some limitations of the flotation method of fecal examination. J. Lab. Clin. Med. 5:386-391.

McFeters, G.A., S.C. Cameron, and M.W. LeChevallier. 1982. Influence of diluents, media and membrane filters on detection of injured waterborne coliform bacteria. Appl. Environ. Microbiol. 43:97-103.

McFeters, G.A., J.E. Schillenger, and D.G. Stuart. 1978. Alternative indicators of water contamination and some physiological characteristics of heterotrophic bacteria in water. In Evaluation of the microbiology standards for drinking water, ed. C.W. Hendricks. U.S. Environmental Protection Agency, Report. No. EPA-570-0-002, Washington, D.C.

McGauhey, P.H. and R.B. Krone. 1967. Soil mantle as a wastewater treatment system. U. of California. SERL Report No. 67-11.

McKinney, R.E., H.E. Langley, and H.D. Tomlinson. 1958. Survival of Salmonella typhosa during anaerobic digestion. I: Experimental methods of high-rate digester studies. Sew. Ind. Wastes. 30:1467-1477.

Miller, R.H. 1973. Soil as a biological filter. In Recycling treated municipal wastewater and sludge through forest and cropland, ed. W.E. Sopper and L.T. Kardos. Penn. State University Press.

Miller, R.H. et al. 1979. Land application of sewage sludge. Ohio Ag. Res. Dev. Center. Research Bulletin 1079 (revised).

Miller, R.H. 1974. Microbiology of sludge disposal in soil. U.S. Environmental Protection Agency, Cincinnati, Report No. EPA-670/2-74--74.

Morrison, S.M. and K.L. Martin. 1976. Pathogen survival in soils receiving waste. In Land as a waste management alternative, ed. A. Loehr. Ann Arbor Science Publishers, Ann Arbor, Michigan, pp. 371-389.

Muller, G. 1953. Untersuchungen uber die Lebersdauer von Askarideneirere in Gardenerde. Zb. Bakteriol. 159:377-379.

Muller G. 1955. Pollution of irrigated grass with bacteria of the typhoid paratyphoid group. Komm. Wirtschaft 8:109.

Napolitano, P.J. and D.R. Rowe. 1966. Microbial content of air near sewage treatment plants. Water Sew. Works 113:480-483.

Newton, W.L., H.J. Bennett, and W.B. Figgatt. 1949. Observations on the effects of various sewage treatment processes upon eggs of _Taenia saginata_. Am. J. Hyg. 49:166-175.

Norman, N.H. and P.W. Kabler. 1954. Bacteriological study of irrigated vegetables. Sew. Ind. Wastes 25:605-609.

Novak, W. 1956. The problem of too wide-spread use of sewage spraying. Gesundheitsing 76:235.

O'Grady, M.R. and J.O.D. Slocombe. 1980. An investigation of variables in a faecal flotation technique. Canada. J. Comp. Med. 44:148-154.

Oldham, W.K. 1973. Spray irrigation of sewage treatment plant effluent at Vernon, British Columbia. Prepared for Okanagon Study Committee in connection with Canada-British Columbia Okanagon Basin Agreement.

Oldham, W.K. 1980. Land application of secondary effluent. Nutrient Removal Seminar; Calgary, Alberta; February 7-8, 1980.

Owen, R.R. and W. Crewe. 1979. The transmission of bovine cysticerciasis in Great Britain. Trans. Royal Soc. Trop. Med. Hyg. 73:324.

Palmieri, J.R. and F.L. Andersen. 1979. Parasites in dog feces. Carolina Tips 42:41-42.

Parsons, D. et al. 1975. Health aspects of sewage effluent irrigation. Pollution Control Branch, British Columbia Water Resources Service, Victoria, B.C.

Pelczar, M.J., R.D. Reid, and E.C.S. Chan. 1977. Microbiology, 4th ed. McGraw-Hill.

Peterson, M.L. 1971. Pathogens associated with solid waste processing. U.S. Environmental Protection Agency, Cincinnati. Progress Report No. SW-49R.

Peterson, M.W. and F.J. Stutzenberger. 1969. Microbiological evaluation of incinerator operations. Appl. Microbiol. 18:8-13.

Piening, H. 1956. Hay polluted with fecal waste waters -- cause of the so-called calf typhoid. Stadehyg. 6:180.

Poon, D. 1966. Studies on the instantaneous death of airborne Escherichia coli. Am. J. Epidemiol. 841.

Popp, L. 1960. The hygiene of agricultural utilization of sewage. Wass. U. Bowden 9:449.

Pratt, P.F. et al. 1978. Removal of biological and chemical contaminants by soil systems in assocation with groundwater recharge by spreading or injection of treated municipal waste water. In State of California, State Water Resources Control Board, Health aspects of waste water recharge. A state-of-the-art review. Water Inf. Center, Inc., Huntington, N.Y.

Prestwood, A.K. 1980. Disease transmission by wild animals from sludge-amended land. In Sludge - Health risks of land application, ed. G. Bitton et al. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Rabinowitz, S.B. 1954. The survival of coliforms, fecal streptococci and Salmonella in soil and climate of Israel. Bull. Res. Counc. Israel 4:205.

Ramirez, A. and J.F. Malina. 1980. Chemicals disinfect sludge. Water Sew. Works 127:52-54.

Rand, M.C. et al., eds. 1976. Standard methods for the examination of water and wastewater. 13th Ed. Am. Pub. Health Assoc., Washington, D.C. 1193 pp.

Rao, N.U. 1973. Removal of pathogenic microorganisms from sewage. Indian Association Water Pollution Control Comm. 17-89.

Ray, B. and M. Speck. 1973. Discrepancies in the enumeration of *E. coli*. Appl. Microbiol. 25:494-498.

Reed, S.C. 1972. Wastewater management by disposal on the land. U.S. Army Corps of Engineers, Cold Regions Res. Eng. Lab. Special Report 171.

Reitter, R. and R. Seligmann. 1947. *Pseudomonas aeruginosa* in drinking water. J. Appl. Bact. 20:145-150.

Reneau, R.B. and D.E. Petty. 1975. Movement of coliform bacteria from septic tank effluent through selected coastal plain soils of Virginia. J. Env. Qual. 4:41-44.

Riemers, R.S. et al. 1979. Investigation of parasites in southern sludges and disinfection by standard sludge treatment processes. U.S. Environmental Protection Agency, Cincinnati. Report No. R-805107-01.

Robens, J.F. 1980. Regulatory aspects of sludge application to land. *In* Sludge - health risks of land application, ed. G. Bitton et al. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Roper, M.M. and K.C. Marshall. 1977. Lysis of *Escherichia coli* by a marine myxobacter. Microb. Ecol. 3:167-171.

Roper, M.M. and K.C. Marshall. 1978. Biological control agents of sewage bacteria in marine habitats. Austrl. J. Mar. Freshwater Res. 29:335-343.

Rudolfs, W., L.L. Falk, and R.A. Ragotzkie. 1950. Literature review on the occurrence and survival of enteric, pathogenic and related organisms in soil, water, sewage and sludge on vegetation. II: Animal parasites. Sew. Ind. Wastes. 22:1417-1427.

Rudolfs, W., L.L. Falk, and R.A. Ragotzkie. 1951a. Contamination of vegetables grown in polluted soil. III:Field studies on Ascaris eggs. Sew. Ind. Wastes. 23:656-660.

Rudolfs, W., L.L. Falk, and R.A. Ragotzkie. 1951b. Contamination of vegetables grown in polluted soil. V: Helminthic decontamination. Sew. Ind. Wastes 23:853-860.

Rudolfs, W., L.L. Falk, and R.A. Ragotzkie. 1951c. Contamination of vegetables grown in polluted soil. VI: Application of results. Sew. Ind. Wastes 23:992-1000.

Rylander, R. 1978. Exposure to Gram-negative bacteria and the development of byssinosis. XIX International Conference of Occupational Health, Dubrovnik, Yugoslavia.

Rylander, R. et al. 1976. Sewage workers syndrome. Lancet 28:478-479.

Rylander, R. et al. 1979. Local and systemic immune response to cotton dust bacteria. 3rd Special Session on Cotton Dust, the Beltwide Cotton Conference. Phoenix, Arizona.

Sabey, B.R., J. Danford, and H.H. Champlin. 1973. Denver sludge recycled on land. Compost Science 14:4-6.

Sagik, B.P., Duboise, S.M. and C.A. Sorber. 1980. Health risks associated with microbial agents in municipal sludge. In

Sludge - health risks of land application. ed. Bitton et al. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Sattar, S.A. In press. Viruses and land disposal of sewage sludge: a literature review. In Biological health risks of sludge disposal to land in cold climates, ed. P.M. Wallis and D.L. Lehmann. University of Calgary Press.

Scheraga, M., M. Meskill, and C.D. Litchfield. 1979. Analysis of methods for the recovery of bacteria sorbed onto marine sediments. In Methodology for biomass determinations and microbial activities in sediments, ed. C.D. Litchfield and A. Seyfried. Am. Soc. Testing & Materials, Special Technical Publication No. 673, Philadelphia, Pa.

Schmauder, G. and H. Unger. 1960. Results of bacterial counts on meadow plants when sprayed with sewage. Wasser.-Wassertech. 10:500.

Scholtan, T. and J. Yang. 1975. Parasite findings at the Toronto Public Health Laboratory over the period 1964 - October, 1974. Abstract. Canadian Journal of Pub. Health. 66:50.

Schusner, D.L., F.F Busta, and M.L. Speck. 1971. Inhibition of injured E. coli by several selective agents. Appl. Microbiol. 21:46-49.

Seabrook, B.L. 1973. Irrigating with liquid digested sludge. Compost Science 24:26-27.

Sekla, L.H. 1970. Some facets of human salmonellosis in Manitoba. Can. J. Pub Health 61:141-148.

Sepp, E. 1971. The use of sewage for irrigation -- a literature review. State of California Dept. Pub. Health, Bureau of Sanitary Engineering.

Shimwell, D.W. 1971. The description and classification of vegetation. U. of Washington Press, Seattle.

Sivinski, H.D. and W.J. Whitfield. 1975. Thermoradiation treatment of a sludge to eliminate pathogens for safe use as fertilizer and animal food supplement. Sandia Laboratories, Albuquerque, New Mexico. Sand. 75-5640.

Smith, W. 1961. The development of the bacterial flora of the faeces of animals and man: The changes that occur during aging. J. Appl. Bact. 24:235-241.

Sopper, W.E. and L.T. Kardos. 1973. Recycling treated municipal wastewater and sludge through forest and cropland. Penn State U. Press, University Park, Pa.

Sorber, C.A., S.A. Schaub, and K.J. Guter. 1972. Evaluation of health and hygiene aspects of land disposal of wastewater at military installations. U.S. Army Medical Environment Eng. Research Unit. Edgewood Arsenal, Maryland.

Soulsby, E.J.L. 1965. Textbook of veterinary clinical parasitology. Oxford: Blackwell.

Soulsby, E.J.L. 1968. Helminths, arthropods and protozoa of domesticated animals. Baillerie, Tindall and Cassell, London.

Spindler, L.A. 1929. On the use of a method for the isolation of _Ascaris_ eggs from the soil. Am. J. Hyg. 10:157-164.

Stone, R. 1953. Land disposal of sewage and industrial wastes. Sew. Ind. Wastes 25:231-238.

Sutter, V.L., V. Hurst, and C.W. Lane. 1967. Quantification of _Pseudomonas aeruginosa_ in faeces of healthy human adults. Health Lab. Sci. 4:245-249.

Tatum, H.W., W.H. Ewing, and R.E. Weaver. 1974. Miscellaneous gram negative bacteria. *In* Lennette and Spaulding Manual of Clinical Microbiology, 2nd ed. Am. Soc. Microbiol. Bethesda.

Taylor, J. 1966. Public health aspects of salmonellosis. *In* Some diseases of animals transmitted to man, ed. Graham-Jones.

Taylor, R.J. and M.R. Burrows. 1971. The survival of *Escherichia coli* and *Salmonella dublin* in slurry on pasture and the infectivity of *S. dublin* for grazing calves. Brit. Vet. Journal 127:530-543.

Temple, K.L., A.K. Camper, and G.A. McFeters. 1980. Survival of two Enterobacteria in feces buried in soil under field conditions. Appl. Environ. Microbiol. 40:794-797.

Thunegard, E. 1975. On the persistence of bacteria in manure. Acta Vet. Scand. Suppl. 56:1-86.

Tracy, H.W., Camarena, V.M. and F. Wing. 1966. Coliform persistence in highly chlorinated waters. J.Am. Water Works. Assoc. 58:1151-1159.

Trout, T.J. and D.B. McWhorter. 1975. Environmental effects of land application of digested municipal sludge. Res. Applied to National Needs, National Science Foundation, Washington, D.C., Report. No. NSF - RA - 186.

Trout, T.J., J.L. Smith, and D.B. McWhorter. 1975. Environmental effects of land application of digested municipal sludge. Res. Applied to National Needs. National Science Foundation, Washington, D.C. Report No. NSF-RA-E-75-186.

United States. Environmental Protection Agency. 1974. Process design manual for sludge treatment and disposal. EPA Report No. 625/1-74-0006.

Van der Drift et al. 1977. Removal of _Escherichia coli_ in wastewater by activated sludge. Appl. Environ. Microbiol. 34:315-319.

Van Donsel, D.S., E.E. Geldreich, and N.A. Clark. 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to storm water pollution. Appl. Microbiol. 15:1362-1370.

Van Fleet, G.L. 1973. Phosphorus removal in Ontario. Paper presented at Canada-Ontario Agreement Seminar:"Phosphorus Removal Design Seminar." Toronto, May 1973.

Van Kleeck, L.W. 1958. Do's and dont's of using sludge for soil conditioning and fertilizing. Wastes Eng. 29:256-257.

Water Pollution Control Federation. Manual of Practice, No. 2. 1972. Utilization of municipal wastewater sludge. Technical Practice Committee, Subcommittee on Utilization of Municipal Wastewater Sludge.

Walker, J., B. Carbonnelle, and H. LeClerc. 1977. Auto-Epuration microbienne par Langunage. Water Res. 11:17-29.

Wang, W.L. and S.G. Dunlop. 1954. Animal parasites in sewage and irrigation water. Sew. Ind. Wastes. 26:1020-1032.

Wheater, D.W.F. et al. 1980. _Pseudomonas aeruginosa_ and _Escherichia coli_ in sewage and fresh water. Water Res. 14:713-721.

Wiley, J.S. 1962. Pathogen survival in composting municipal wastes. J. Water Poll. Control Fed. 34:80-90.

Wiley, B.B. and S.C. Westerbrook. 1969. Survival of human pathogens in composted sewage. Appl. Microbiol. 18:994.

Wilson, C.W. and F.E. Beckett. 1968. Municipal sewage effluent for irrigation. Louisiana Tech. Alumni Foundation, Huston, Louisiana.

Wood, G.W. et al. 1975. Faunal response to spray irrigation of chlorinated sewage effluent. Institute Res. Landwater Resources. Penn State University.

World Health Organization. 1967. Control of Ascariasis. World Health Organization, Geneva, Tech. Rept. Ser. No 379.

World Health Organization. 1981. The risk of health of microbes in sewage sludge applied to land. World Health Organization, EURO Reports and Studies 54.

Wray, C. 1975. Survival and spreading of pathogenic bacteria of veterinary importance within the environment. Vet. Bull. 45:543-550.

Zaske, S.K., W.S. Dockins, and G.A. McFeters. 1980. Cell envelope damage in _Escherichia coli_ caused by short-term stress in water. Appl. Environ. Microbiol. 40:386-390.